Dublin City University

School of Health and Human Performance

Project Submission Form

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This dissertation is submitted in fulfilment of the requirements for a MSc. Degree in Exercise Physiology at the School of Health and Human Performance in Dublin City University.

Declaration

I hereby certify that this material, which I now submit for assessment on the programme of study leading to the award of MSc. in Exercise Physiology is entirely my own work and has not been taken from the work of others save and to the extent that such work has been cited and acknowledged within the text of my work.

I would like to declare that the insulin measurements were conducted with the assistance of Mr. Declan Gasparro at St. James' Hospital, Dublin and the non-esterified fatty acids with Mr. Michael Harrison at Waterford Institute of Technology.

Signed: Fiona Sarsfield

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Publications

The following published research has arisen, to date, from the information contained in this thesis:

- Sarsfield FM, Egan B, O'Connor P, O'Brien K, Moyna NM, McCaffrey N, O'Gorman DJ. The Impact of Carbohydrate Ingestion on the Regulation of Fat Oxidation Following Exercise. Medicine & Science in Sports & Exercise 2006 May; 38(5): S542

Some of the information contained in this thesis may also be prepared for publication at a later date

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The Impact of Carbohydrate Ingestion on the Regulation of Fat Oxidation Following Exercise

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Purpose: To determine the impact of carbohydrate (CHO) ingestion on the regulation of fat oxidation following glycogen lowering exercise.

Methods: Six males (age 23.4 ± 1.7y, $\dot{V}O_{2peak}$ 58.0 ± 2.1 $ml \cdot kg^{-1} \cdot min^{-1}$) participated in the study. Subjects performed an incremental maximal cycle ergometer test and a verification trial to determine the workload corresponding to 75% $\dot{V}O_{2peak}$. On separate days, subjects exercised at 75% $\dot{V}O_{2peak}$ for 90 min. Following exercise, subjects consumed either a CHO drink or a flavoured placebo (PLA). Substrate oxidation was measured for 1 h after exercise using indirect calorimetry. Before, immediately after, and 1 h after exercise, subjects had a muscle biopsy taken from the vastus lateralis.

Results: Subjects exercised at a similar % $\dot{V}O_{2peak}$ during both the CHO (71.5 ± 1.8% $\dot{V}O_{2peak}$) and the PLA (72.5 ± 1.8% $\dot{V}O_{2peak}$) trials. Energy expended during and following exercise was similar between trials. The contribution of fat towards energy expenditure (%EE) during recovery was lower in the CHO trial than in the placebo trial ($p<0.05$). The opposite was true for carbohydrate ($p<0.05$). Exercise lowered muscle glycogen in both trials. Exercise decreased ($p<0.05$) serum insulin levels and increased ($p<0.05$) non-esterified fatty acid (NEFA) levels. CHO ingestion led to a dramatic increase in glucose and insulin levels and a drop in NEFA levels ($p<0.05$). The phosphorylataion of acetyl-CoA carboxylase (ACC), 5'AMP-activated protein kinase (AMPK) and hormone sensitive lipase (HSL) increased with exercise ($p<0.05$). ACC and AMPK phosphorylation in the CHO trial was lower at 1 h post than in the PLA trial ($p<0.05$). HSL phosphorylation remained above baseline at 1 h post in the PLA but not the CHO trial.

Conclusion: Results indicate that CHO ingestion following exercise suppressed fat oxidation, possibly by relieving the phosphorylation induced inhibition of ACC, allowing for an increase in malonyl CoA levels.

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List of Abbreviations

BMI	Body Mass index
$\dot{V}O_{2peak}$	Peak Oxygen Uptake
CHO	Carbohydrate
FFA	Free Fatty Acid
TG	Triglyceride
VLDL	Very Low Density Lipoproteins
IMTG	Intramuscular Tyiglycerides
AMPK	5'AMP-Activated Protein Kinase
ACC	Acetyl-CoA Carboxylase
HSL	Hormone Sensitive Lipase
NEFA	Non-Esterified Fatty Acid
RER	Respiratory Exchange Ratio
β	Beta
CoA	Coenzyme
CPTI	Carnitine Palmitoyltransferase I
cAMP	Cyclic Adenosine Monophosphate
PKA	Protein Kinase A
Ser	Serine
PKC	Protein Kinase C
ERK	Extracellular Signal Regulated Kinase
AICAR	5-Aminoimidazole-4-Carboxamide-Riboside
SNS	Sympathetic Nervous System
DAG	Diacylglyceride
$FABP_{PM}$	Fatty Acid Binding Protein
FAT/CD36	Fatty Acid Translocase
FATP	Fatty Acid Transport Protein

Glossary of Terms

Physical Activity

Any form of muscular movement, ranging from everyday activities to sports

Exercise

Physical activity that is a planned, structured movement of the body designed to enhance health and fitness

Metabolic Related Disorders

Illnesses or abnormal functioning related to metabolism or metabolic system

Homeostasis

The maintenance of stability and consistency in the internal environment

Adipokines

Peptide hormones and cytokines produced and secreted by adipocytes

Oxidise

To combine, or cause an element or radical to combine, with oxygen or to lose an electron

Exercise Intensity

The level of exertion required to sustain exercise. It is usually determined by the force generated, the velocity of movement or the percentage or maximal ability.

Exercise Duration

The length of time for which exercise continues

Recovery

The period following exercise during which all physiological parameters return to their pre-exercise state

Isocaloric Exercise

Exercise sessions during which the same number of calories are expended

At Rest

Non-exercising state

Relative Exercise Intensity

Exercise intensity as a percentage of an individual's peak oxygen uptake

Peak Oxygen Uptake ($\dot{V}O_{2peak}$)

The peak amount of oxygen taken in by the lungs, transported in blood around the body and used by tissues in the body per minute during exercise

Respiratory Exchange Ratio

The ratio of the net output of carbon dioxide to the simultaneous net uptake of oxygen

Endurance Training

Repeated bouts of submaximal aerobic exercise completed over a number of weeks, months or years.

Diet

The sum of the food consumed by an organism. May also refer to regular eating habits

Endogenously

Originating from within the body

Exogenously

Originating from outside the body

Phosphorylation

The introduction or addition of a phosphate group into an organic molecule

Dephosphorylation

Removal of a phosphate group from an organic compound

Passive Diffusion

The movement of a substance down a concentration gradient. This may often occur across a cell membrane and does not require energy

Facilitated Transport

A form of passive transport where molecules cross membranes with the assistance of transport proteins

Submaximal Running

Running that does not require maximal effort

Flux

The total amount of a quantity passing through a given surface per unit time

Experimental Trial

A period during which experimental data is collected

Heavy Physical Activity

Any form of muscular movement requiring a lot of effort

Chapter 1: Introduction

Introduction

All living cells require energy, not only to sustain viability, but to facilitate any physiological processes to which they may contribute. In particular, physical activity requires the coordinated response of cells, tissues and organs to produce energy to generate force for movement. The sum of the chemical reactions that contribute to energy production in the body is referred to as metabolism (1).

The majority of energy produced by the body, in the form of adenosine triphosphate (ATP), occurs under aerobic conditions in the mitochondria. However, cells also store small amounts of ATP and can quickly generate more ATP in the absence of oxygen, by using creatine phosphate to generate ATP or through anaerobic glycolysis. Anaerobic energy production is less efficient and cannot be sustained for prolonged periods of time (1).

The production of ATP occurs following the catabolism of endogenous and exogenous substrates. While these substrates can be present in many different forms, as discussed later, they can be broadly categorized as carbohydrate (CHO), fat and protein macronutrients (1).

Macronutrients for Energy Production

CHO exist as monosaccharides, disaccharides and polysaccharides. Monosaccharides and disaccharides are also known as simple sugars while polysaccharides are referred to as complex carbohydrates (1). Upon ingestion, disaccharides and polysaccharides are broken down into monosaccharides which are then absorbed by the small intestine. Absorbed monosaccharides travel to the liver where they are converted to glucose. Glucose is then either stored in the liver as

glycogen or released into the blood. Blood glucose may then be taken up by the muscle and stored as intramuscular glycogen (1). Liver and muscle glycogen stores are primarily used for energy. If liver and muscle glycogen stores are full, excess glucose can be converted to fat by the liver and stored in adipose tissue as energy (1).

Lipids, or fats, represent the predominant fuel source while at rest and during light to moderate activity (1). Lipids are available both endogenously and exogenously. Exogenous lipids are primarily derived from the diet in the form of triglycerides (TG). Following ingestion, TG are broken down by enzymes called lipases into two free fatty acids (FFA) and one monoglyceride. FFA readily aggregate to form micelles until they are taken up individually by the enterocytes (1). FFA inside the enterocytes are then reesterified into TG, and packaged with proteins and phospholipids to form chylomicrons. Chylomicrons enter the circulatory system at the thoracic duct. Lipoprotein lipase, an enzyme found attached to endothelial cells in adipose tissue, skeletal and cardiac muscle and liver, liberates FFA from chylomicrons. FFA are then taken up by specific tissues. In adipose tissue, FFA are predominantly reesterified and stored as TG. In skeletal muscle, FFA are mainly oxidised, however, small amounts are also stored intramuscularly as TG (1).

Protein serves a variety of physiological functions in the human body. They are the building blocks of all human tissue and form a vital part of cell nuclei, membranes and all enzymes. Although it has been traditionally thought that the role of protein in providing energy is not important, it is becoming increasingly clear that its contribution may be quite significant. Recent research has suggested that protein may contribute between 5% and 10% of energy needs at rest and up to 15% during exercise (1). Proteins are broken down into amino acids following consumption. Excess amino acids

are not stored in the body. Instead, the amine group is removed and excreted and the remainder of the molecule converted to glucose, glycogen or fat and stored as energy (1). Some amino acids have important implications for gluconeogenesis and nitrogen balance during and following exercise. Glutamine, glutamate and alanine are gluconeogenic precursors that interlink with pyruvate metabolism and are therefore essential to carbohydrate metabolism (2). An extensive discussion of protein metabolism at rest and during exercise is beyond the scope of this project and for this reason the following chapters will focus only on the contribution of carbohydrate and fat to energy expenditure during and following exercise and the metabolic pathways regulating each.

The Key Role of Adipose Tissue-Liver-Skeletal Muscle Interactions in Metabolism

Whole-body metabolism does not happen in isolation but requires integration across tissues (2). All tissues require energy but the over-consumption of energy-dense foods and reduced physical activity leads to changes in the metabolic function of skeletal muscle, liver and adipose tissue; which have been identified as the major tissues involved in metabolism (3). There is increasing evidence to support a hierarchical response from these tissues in the human body. It is thought that adipose tissue and the liver may act as primary or "driver" organs for systemic metabolic regulation and dysregulation, whereas skeletal muscle may serve as a secondary or "responder" tissue (3). The highly coordinated integration of these tissues is essential to energy provision at rest and during exercise, and to the reinstatement of homeostasis during recovery from exercise (2).

Although once considered a passive tissue for energy storage, adipose tissue is now recognized as an important endocrine organ that informs the brain and peripheral

tissues of changes in whole-body energy status (3, 4). Recent research has exposed a group of circulating adipokines that signal changes in energy status centrally and to other metabolic organs (3). Adipose tissue secretes leptin which has a controlling effect on satiety and hunger and which has been linked to catecholamines and insulin, thereby affecting lipid and CHO metabolism (4). Adipokines released by adipose tissue can also affect insulin and increase lipolysis. Adipose tissue is responsible for the increased release of lipids witnessed during exercise, an effect which is influenced by hormone sensitive lipase (HSL) (5).

The liver also plays a driving role in the regulation of metabolism (3). Glucose is produced by glycogenolysis and gluconeogenesis in the liver, processes which are influenced greatly by concentrations of glucagon and insulin (6, 7). In the fed state, the liver helps to distribute lipids by esterification of FFA into TG and packaging into very low density lipoproteins (VLDL) for export into the circulation and ultimate storage of FFA in the adipose triglyceride pool (3). During exercise, the liver is central to the maintenance of glucose delivery to skeletal muscle. Liver glucose production is activated along with reduced insulin, increased glucagon and catecholamines and improved availability of gluconeogenic precursor's glutamine and alanine (8).

Skeletal muscle responds to the actions made by adipose tissue and the liver in response to a number of situations including exercise, lipid overload and starvation (3). The metabolic processes and interactions involving adipose tissue, the liver and skeletal muscle are complex and for this reason the focus of this research project will be primarily on the key metabolic pathways regulating CHO and fat oxidation at the level of the muscle. The regulation of fat and CHO metabolism is under the influence of a number of signaling cascades. Several regulatory sites have been proposed included

regulation by 5'AMP-activated protein kinase (AMPK), mediated in part by its effects on hormone sensitive lipase (HSL) and acetyl-CoA carboxylase (ACC).

Exercise and Substrate Metabolism

Exercise plays an important role in the regulation of substrate metabolism (9). Understanding fat oxidation during and following exercise and its interactions with CHO metabolism may help in our understanding of training adaptations and devising exercise recommendations for increasing fat oxidation and therefore playing a role in the treatment and prevention of overweight and obesity (9, 10). Although protein is increasingly recognized as a significant fuel provider, CHO and fats remain the predominant fuels used for energy at rest, during exercise and during recovery from exercise.

Exercise increases energy demands both during and following exercise, leading to increased lipid oxidation during these periods (11, 12, 13, 14). The extent to which fat oxidation is increased is dependent on a number of factors, including both the intensity and the duration of the exercise. It is well known that changes in exercise intensity induce changes in substrate utilization (15, 16). Low to moderate intensity exercise ($\leq$ 65%peak oxygen uptake ($\dot{V}O_{2peak}$)) results in the highest rate of fat oxidation. With exercise intensities above 65% $\dot{V}O_{2peak}$, there is a subsequent shift towards CHO as the main source of energy (15, 16). It has also been established that the contribution of lipids to total energy expenditure increases with increasing exercise duration. This is thought to be due to the progressive reduction in muscle glycogen stores witnessed during prolonged exercise (9).

Sources of lipids include FFA and TG found in plasma, and TG stored in adipose tissue and intramuscularly. It has been reported that at the onset of low intensity exercise, plasma FFA oxidation accounts for up to 80% of total energy expenditure, with only a very small percentage derived from other fat sources and CHO (8, 17, 18). As exercise continues, the relative contribution of intramuscular triglycerides (IMTG) to total fat oxidized increases up to 2 hrs of exercise before slowly starting to decrease. Controversy however surrounds the use of IMTG during exercise and recovery. Whereas isotope tracer and ^{1}H magnetic resonance spectroscopy studies have demonstrated IMTG use during and after exercise, studies incorporating muscle biopsies often report no statistical differences between pre and post exercise biopsies (19). Research into the use of IMTG during exercise and recovery has grown in importance due to the observed correlation between an increased IMTG content and insulin resistance (20).

Although there is a tendency to consider the effects of exercise as limited solely to the exercise period, the recovery and post-exercise phase constitutes a much longer period of time and can result in major cumulative effects on substrate oxidation (21). Although the intensity and duration of exercise play a significant role in determining fuel selection in the post-exercise period, evidence indicates that the total energy expended during a bout of exercise is perhaps the most important factor with regards to substrate metabolism following exercise (22). Isocaloric exercise of varying intensity has been shown to elicit similar rates of fat oxidation over a 6 h recovery period (10).

Research clearly indicates increased lipid oxidation following exercise, and this phenomenon has been shown to continue despite CHO feeding, during or after exercise (22). It is thought that following glycogen depleting exercise, CHO is preferentially

partitioned towards the restoration of muscle glycogen stores and so all gluconeogenic precursors are spared from oxidation and instead trafficked towards storage (23, 24, 25, 26). Glycogen replenishment is of metabolic priority and fat used for energy needs.

Rationale

The regulation of fat oxidation during and following exercise is not fully understood. The majority of available research has focused on investigating the impact of CHO, ingested before or during exercise, on fat metabolism. Little is known about the effect of CHO ingestion in recovery from exercise, on the regulation of fat oxidation in the post exercise period. Determining the impact of CHO ingestion following exercise will help in our understanding of fat metabolism and could help to devise recommendations to increase fat use.

It is well recognized that alterations in fat metabolism play an important role in the development of metabolic related disorders and cardiovascular disease (27, 28). Recent evidence has also shown a significant correlation between increased IMTG levels and insulin resistance (28, 29). It is apparent that the up regulation of lipid oxidation during and after exercise is central to increasing and maintaining weight loss and essential for good health (10). Understanding the factors, including the signaling pathways, that regulate FFA oxidation during and following exercise is therefore of great clinical importance (30). The pathways concerning AMPK and the regulation of CHO and fat metabolism are of particular interest as they have recently emerged as key regulators of substrate utilization and as yet have not been extensively studied in the post exercise period. Although alternative pathways, such as those concerning fatty acid translocase (FAT/CD36), glycerol-3-phosphate acyltransferase (GPAT), malonyl-CoA decarboxylase (MCD), and others, are also thought to play a significant role, the

primary focus of this study was placed on AMPK.

Aim of the Research

1. To investigate the role of the AMPK signaling cascade in skeletal muscle on the regulation of whole body fat oxidation in the post exercise period, with and without carbohydrate ingestion

Objectives

1. To determine the effect of carbohydrate supplementation on the rate of whole body fat oxidation in the hour following glycogen lowering exercise
2. To determine the effect of carbohydrate supplementation on the phosphorylation of AMPK and downstream targets prior to, immediately following and 1 h post exercise.

Study Hypothesis

1. In the hour following glycogen lowering exercise, the rate of whole body fat oxidation will not be increased above baseline. (Objective 1).
2. The ingestion of a high dose carbohydrate solution will have no effect on the rate of whole body fat oxidation. (Objective 1).
3. There will be no difference in the AMPK signaling cascade following glycogen lowering exercise, with or without carbohydrate ingestion. (Objective 2)

Limitations of the Study

1. The measurements of whole body oxidation rates were based on non-protein respiratory quotient (RQ) results; therefore protein oxidation was not determined in this study. This is based on the assumption that the contribution of protein oxidation to energy production during exercise of this nature is minimal.
2. This study examines one metabolic pathway regulating substrate utilization, but it must be acknowledged that many factors may influence fuel selection.
3. The wider endocrine regulation of metabolism (eg. growth hormone, cortisol, epinephrine, etc) was not determined in this research project.
4. The small subject sample size may limit the statistical power of the results

Delimitations of the Study

1. The results of the study are delimited to male adults between 18-35 years of age and so they may not directly apply to a wider population
2. The protocol employed was a prolonged bout of moderate-to-high intensity exercise and may not reflect other exercise challenges.
3. There was a large amount of high concentration carbohydrate supplied following the exercise trial that may not be reflective of a more physiological dose

Chapter 2: Review of Literature

Introduction

The majority of ATP produced during prolonged exercise is via the mitochondrial oxidation of fat and CHO. In the absence of exogenous nutrient ingestion during or after exercise, the body must rely on (i) lipids, either stored in adipose tissue and skeletal muscle or circulating FFA and TG and (ii) CHO, stored as muscle and liver glycogen or circulating glucose. Body fat stores are abundant and represent 92-98% of all endogenously stored energy, with CHO contributing only about 2-8% (31). While the availability of lipid energy stores far exceeds CHO, the latter is preferential for oxidation and is rate limiting for prolonged endurance exercise. The mechanisms underlying the shift from lipid to CHO oxidation during exercise or the post exercise regulation of substrate use is poorly understood. However, a number of factors can alter the substrate utilization rates of CHO and lipid to meet the increased energy demands of exercise including the intensity, duration, and type of exercise (i.e. endurance or resistance), the training status of the individual and macronutrient ingestion (21). This review will explore the various factors that influence substrate utilization in skeletal muscle in response to exercise and present the current understanding of the mechanisms involved.

The Influence of Exercise Intensity and Duration on Substrate Utilisation

It has been demonstrated that exercise at an intensity of $\leq$65% $\dot{V}O_{2peak}$ relies primarily fat oxidation whereas exercise of greater intensities relies on CHO as the primary substrate (21). Romijn et al. (17) and others (32) have reported that upon the onset of exercise at 25% $\dot{V}O_{2peak}$, plasma FFA oxidation accounted for >80% of the total energy expenditure and 100% of lipid metabolism. During moderate intensity exercise, 65% $\dot{V}O_{2peak}$, the contribution of plasma derived FFA to total energy expenditure

decreases and the contribution of FFA from intramuscular lipolysis increases (8). Fat oxidation accounts for approximately 40% of the energy used during exercise at this intensity (9, 33). At high intensity exercise, 85%$\dot{V}O_{2peak}$, IMTG becomes the major source of lipids for oxidation; however, there is now a preponderance of CHO oxidation, its primary source being muscle glycogen (9, 33). The rate of fat oxidation during intermittent high intensity exercise has been reported to be 3-fold lower (0.1 g/min) than during continuous lower intensity exercise (0.3 g/min) (34). The intensity dependent shift in substrate utilization was used to develop the concept of substrate crossover. The "crossover point" (Figure 1), a term coined by Brooks in the 1990's (35), describes the relative exercise intensity at which the predominant fuel for substrate metabolism shifts from fat to CHO.

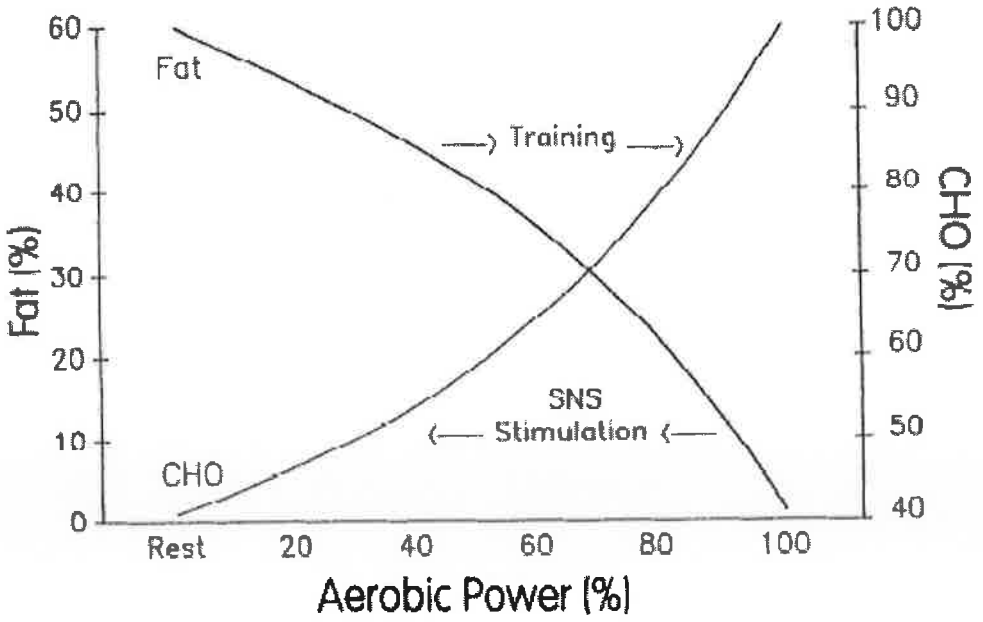


Figure 1: Brooks et al. (35), Relative Contribution to Substrate Oxidation at Rest and During Exercise: Crossover Concept. (Adapted from Brooks and Mercer, 1994)

It has also been observed that fat oxidation is enhanced as exercise duration increases (36). This has been attributed to the decrease in muscle glycogen concentrations and therefore the availability of CHO for ATP production. As a result there is a progressive rise in plasma FFA concentration and an increase in IMTG lipolysis (9).

There has been relatively little research examining the impact of exercise intensity and duration on post-exercise substrate utilization. The available evidence would suggest that the most important factor in understanding post exercise substrate oxidation is the total energy expended during exercise (37, 38, 39). Isocaloric aerobic exercise of differing intensities and durations has been shown to elicit similar rates of fat oxidation during a 6 h recovery period (10).

Substrate Utilization during Endurance and Resistance Exercise

The type of exercise plays an important role in determining the substrates used during exercise (21). Heavy resistance exercise relies predominately on anaerobic glycolysis which utilizes CHO exclusively (40). Endurance exercise relies mainly on the tricarboxylic acid (TCA) cycle and on a mixture of fat and CHO for energy production (21).

In the post exercise phase, fat oxidation has been shown to be higher following resistance exercise than following endurance exercise. Burlesson et al. (41) reported that more fat was oxidized following resistance exercise than following endurance exercise. It has been proposed that this may be due to fuel selection during the exercise bout. Resistance exercise results in greater depletion of muscle glycogen and so it is thought that fat is predominantly used post exercise in order to allow for the

replenishment of the body's glycogen stores (21). Shuenke et al. (42) reported a decrease in respiratory exchange ratio (RER) from 0.89 to 0.79 immediately after a 31 min resistance exercise bout. RER was still significantly reduced at 0.84, up to 43 h later. Resistance training however does not appear to have the same beneficial effects on fat oxidation as an acute bout of resistance exercise. The increase in fat oxidation following resistance training it thought to be due directly to the last bout of exercise rather than a training effect (15). Several studies report no difference in RER values before and after training (43, 44, 45).

Training Status

Endurance training results in physiological adaptations that enhance fuel mobilization and utilization (33). Both cross-sectional and longitudinal studies have provided evidence of a reduced reliance on CHO as an energy source as a result of endurance training (46, 47, 48, 49, 50, 51, 52, 53). Friedlander et al. (13) showed fat oxidation to be increased during exercise at both the same absolute and relative intensities, following a 12 wk training program undertaken by 8 untrained females. Prior to training, subjects performed an exercise trial at 65% $\dot{V}O_{2peak}$. Subjects then completed two exercise trials upon completion of the training program, one at the same absolute workload as the pre-training trial, and one at the same relative intensity. RER was significantly decreased from 0.91 to 0.88 at the same relative intensity and further decreased to 0.86 at the same absolute intensity (13). Achten & Jeukendrup (54) compared rates of fat oxidation during exercise between moderately trained and well trained cyclists. When cycling at 62% $\dot{V}O_{2peak}$, moderately trained cyclists displayed fat oxidation rates of 0.48 $\pm$ 0.15 g·min^{-1} with rates of 0.56 $\pm$ 0.14 g·min^{-1} in well trained subjects (54). Research has also shown trained individuals to rely more on the

contribution of IMTG to total energy expenditure than untrained individuals (50).

Macronutrient Ingestion and Substrate Utilization in Response to Exercise

Diet is possibly the most potent intervention that alters fuel use during and after exercise (9). Research has shown that consuming a high fat diet before exercise increases the contribution of fat to substrate metabolism. Jansson & Kaijser (47) found that cycling at 65% $\dot{V}O_{2peak}$ elicited an RER response of 0.92 when subjects were fed a high CHO diet, and an RER of 0.81 following consumption of a high fat diet for the previous 7 days. An increase in FFA availability and an adequate supply of oxygen for mitochondrial oxidation can decrease the reliance on the body's liver and muscle glycogen stores (9). Similarly, increased availability and oxidation of CHO results in a decrease in the contribution of fat to energy supply (55, 56, 57). Studies have shown however, that CHO ingestion following exercise does not completely blunt fat oxidation (23, 24, 25, 26). This is possibly explained by the partitioning of glucose towards glycogen repletion rather than oxidation in the recovery phase (23, 24, 25, 26). Thus, in the post exercise period, fat oxidation remains elevated, despite increased availability of CHO.

Cellular Regulation of Substrate Utilization

The regulatory mechanisms responsible for the increase in fat oxidation seen during and post exercise are still not fully understood. Several sites have been suggested at which fat oxidation may be regulated: 1) Adipose tissue and IMTG lipolysis and re-esterification, 2) FFA uptake by the muscle, and 3) FFA movement across the mitochondrial membrane (15, 16). These will now be discussed in more detail.

The Regulation of Lipolysis in Adipose Tissue and Skeletal Muscle

In the absence of exogenously available TG, regulation of the release of FFA from adipose tissue (lipolysis) is thought to be the chief factor determining the circulating concentration of FFA (33). HSL liberates FFA from TG stored in adipose tissue and skeletal muscle (33, 58). HSL is regulated via phosphorylation/dephosphorylation and is under the control of the hormones insulin and epinephrine. The rate of lipolysis is largely determined by the balance between the stimulatory effect of epinephrine and the inhibitory effect of insulin (33).

Epinephrine is the foremost stimulator of lipolysis. When epinephrine binds to its β-adrenergic receptor in the plasma membrane, the membrane-bound enzyme adenylate cyclase is activated. This results in an increase in intracellular cyclic adenosine monophosphate (cAMP) levels. cAMP directly activates cAMP-dependent protein kinase A (PKA), which phosphorylates and activates HSL at residues Serine $(Ser)^{563}$, Ser^{659} and Ser^{660}, although Ser^{563} may not affect catalytic activity directly (59, 60, 61).

Sensitivity to epinephrine can be affected by the physiological energy state of the body. In short-term (3-day) fasting, when the rate of lipid oxidation is increased, the lipolytic responsiveness to epinephrine is enhanced, whereas in obesity, the responsiveness to epinephrine is blunted (37). Exercise has been shown to increase FFA availability, due mainly to increased lipolysis and a decreased rate of reesterification (8). The onset of exercise is associated with a rapid increase in circulating epinephrine and a decrease in plasma insulin. These changes in hormone flux increase the rate of lipolysis and increase blood flow perfusion in adipose tissue. This results in an increase in circulating FFA which become more readily available for

delivery to the muscle (31). Wolfe et al. (62) reported that the percent of FFA reesterified to TG dropped from approximately 70% at rest to approximately 25% during 30 min of low to moderate intensity exercise. The results of this study however are based on measurements of the rate of appearance of glycerol as a measure for whole-body lipolysis. As it has been suggested that this method may lead to an underestimation of the true rate of lipolysis, results should be interpreted with some caution (62). During moderate intensity exercise, lipolysis has been shown to increase approximately 3-fold. This is thought to be largely as a result of elevated β-adrenergic stimulation (4, 31, 63). Blood flow to the muscle is also increased considerably during exercise, thus improving delivery of FFA to the working muscle (31).

It has become apparent that, during exercise, an increase in epinephrine is not essential for an increase in HSL activity (64) and that β-adrenergic-independent signaling cascades can activate HSL. (58, 63, 65). In studies with rats, combined stimulation with epinephrine and contraction portrayed partially additive effects on HSL activity in skeletal muscle (59). This indicates that epinephrine and exercise activate HSL by at least partly different signaling pathways (58). The fact that only partially additive effects were reported refutes the claim that skeletal muscle contains two different isoforms of HSL and instead supports the notion that epinephrine and contraction may activate different kinases, which, in turn, may phosphorylate different sites of the same muscle HSL isoform (58). There is some evidence to indicate that the effect of contraction on HSL occurs at Ser^{600} and is mediated by protein kinase C (PKC), partly via activation of extracellular signal regulated kinase (ERK) (58, 65, 66).

In humans, HSL has been shown to be increased at 60 min of cycle ergometer exercise. A similar response has been found in adrenalectomized, cortisol substituted

patients, with epinephrine infusion during exercise, indicating that epinephrine may be responsible for the HSL activation witnessed late during exercise (67). In contrast to this, Watt et al. (32, 68) reported increased HSL activity after 1 min of low intensity exercise, before epinephrine levels rose, and also, HSL levels decreased at 2 h of exercise regardless of large increases in plasma epinephrine. These results suggest that epinephrine may not be essential to increase HSL activity during exercise.

The molecular mechanisms behind the activation of HSL in human skeletal muscle during exercise remain largely unknown. AMPK is an enzyme activated by a change in energy state as reflected by a change in the AMP/ATP ratio (69). AMP allosterically activates AMPK by stimulating an upstream kinase, AMPK kinase, which then phosphorylates and activates AMPK (70, 71). The binding of AMP to AMPK also renders the kinase a better substrate for its activator, AMPK kinase, and a worse substrate for protein phosphatases that might inactivate it (72, 73). In adipose tissue, it is thought that AMPK inhibits the HSL activation induced by β-adrenergic agents (74, 75). It seems unlikely however that activation of AMPK would inhibit HSL activity in human skeletal muscle during exercise, considering that this would decrease energy provision from IMTG while the primary role of AMPK activation is to accelerate energy-providing pathways and decelerate energy-consuming pathways (76). In studies by Watt et al. (63) however, the increase in HSL activity in skeletal muscle induced by exercise at 70% $\dot{V}O_{2peak}$ was abolished when AMPK activation was high due to low muscle glycogen levels or due to incubation with 5-aminoimidazole-4-carboxamide-riboside (AICAR), an artificial activator of AMPK. Watt et al. (63) concluded that β-adrenergic stimulation of HSL activity in skeletal muscle can be overridden by AMPK activation of HSL on Ser^{565}. It must be noted however that the above study was

conducted without controlling for circulating glucose, FFA and epinephrine levels and so must be interpreted with some caution (63). In a similar study conducted by Roepstorff et al. (76) which controlled for circulating hormones, glucose and FFA, AMPK activation was reported to increase HSL phosphorylation on Ser^{565}. However, this did not result in a corresponding increase in HSL activity, supporting the authors' hypothesis that in human skeletal muscle, HSL phosphorylation on Ser^{565} is not a primary regulator of HSL activity during exercise (76). The authors also report a similar increase in HSL activity during exercise in both the low glycogen and the high glycogen trials, despite markedly higher AMPK activity at 30 min during the low glycogen trial. In contrast to previous studies, this provides evidence that an increase in AMPK activation during exercise does not inhibit HSL activity (76).

Insulin inhibits HSL by dephosphorylation of the enzyme through phosphodiesterase-3-dependent cAMP degradation (59, 77, 78). During moderate intensity exercise, the sympathetic nervous system (SNS) suppresses insulin release, thus preventing any inhibition of HSL and fat oxidation (4). Glucose ingestion is the predominant signal for pancreatic insulin secretion (33). Previous research has repeatedly shown an inhibitory effect of glucose on plasma FFA concentration and the rate of lipolysis. It is thought that this effect is mediated entirely by increased insulin concentrations. In vivo studies at rest have demonstrated that even slight increases in insulin levels have a significant negative impact on lipolysis (79, 80). Horowitz et al. (55) also showed lipolysis to be suppressed following CHO ingestion during exercise and reported that this suppression was sufficient to limit fat oxidation. It has been demonstrated that the suppressive effect of CHO intake on fat oxidation may last for 6 h or more (15). Insulin secretion is suppressed during exercise, helping to explain the increase in fat oxidation seen at the transition from rest to exercise (9). CHO ingestion

during exercise has been shown to increase plasma insulin and decrease plasma epinephrine levels, attenuating the exercise-induced increase in HSL activity (64). Several studies have shown that lipid metabolism is also augmented during recovery from glycogen depleting exercise (81, 82, 83). This is partly associated with the preferential use of CHO for skeletal muscle glycogen resynthesis (84, 85) and also by an increase in the plasma FFA availability due to a reduction in plasma insulin levels and a rise in the level of catecholamines (81, 86).

Insulin has been shown to affect not only lipolysis but also FFA reesterification (15). CHO ingestion provides glycerol phosphate for FFA reesterification and storage as TG (87, 88, 89). In animal studies, the rate of incorporation of FFA into TG has been reported to increase with increasing insulin levels both at rest and during exercise (90, 91, 92). Sidossis et al. (93) demonstrated decreased rates of fat oxidation under hyperglycaemic and hyperinsulinemic conditions, despite no detected change in FFA uptake. This suggests an alternative fate for FFA, such as reesterification into TG (93). Haemmerle et al. (78) provided evidence of impaired TG and diacylglyceride (DAG) hydrolysis in HSL knock-out mice with increased accumulation of DAG in various tissues, including skeletal muscle (78).

TG synthesis (Figure 2) is a multi-step reaction involving the addition of a FFA in the form of acyl-CoA to a glycerol-3-phosphate molecule to form 1-acylglycerol-3-phosphate (lysophosphatidic acid).

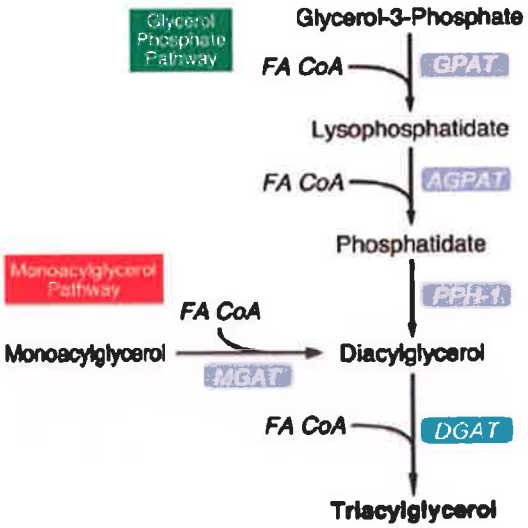


Figure 2: Pathway of Triglyceride Synthesis (adapted from Chen and Farese, 2005 (94))

The initial and rate-limiting step in TG synthesis is catalyzed by the enzyme glycerol-3-phosphate acyltransferase (GPAT) (95). A mitochondrial (mtGPAT) and an endoplasmic reticulum (erGPAT) isoform of GPAT have been identified in humans (95). When energy supply is in surplus following glucose intake, FFA synthesis and TG formation are stimulated in order to store the excess energy (96). The concentration of glucose in the plasma is tightly coupled with the level of insulin release from the pancreas (96). As discussed previously, insulin inhibits lipolysis and promotes FFA reesterification into TG. Increased expression of mtGPAT has been reported in response to increased insulin levels (95).

Diacylglycerol acyltransferase (DGAT) is a membrane-bound enzyme that catalyzes the final step in TG formation by the acylation of diacylglycerol (96). Two

DGAT genes have been identified. DGAT-1 was the first to be sequenced and has been shown to be related to the acyl CoA:cholesterol acyltransferase gene family (96, 97). DGAT-2 is a member of a distinct and independent family (96). DGAT-1 is thought to function in fat absorption and energy homeostasis while DGAT-2 is thought to play a role in TG synthesis and storage (98). DGAT enzymes are hypothesized to be under the dual control of glucose and insulin. Glucose is thought to promote DGAT activity through a preferential increase in DGAT-1 messenger ribonucleic acid (mRNA), whereas insulin is thought to enhance the expression of DGAT-2 mRNA (96). It has been reported that the activity of DGAT is also diminished when AMPK is activated (99).

It was originally thought that FFA availability determined the rate of FFA uptake and oxidation in the mitochondria during exercise. More recent research has shown that FFA uptake displays some evidence of saturation kinetics, with no further increases in FFA uptake despite further rises in plasma FFA concentrations (49, 100, 101). These findings indicate that although the plasma FFA concentration is important for FFA uptake in the muscle, it is likely that other factors inherent in the muscle are important also (102).

Free Fatty Acid Uptake by the Muscle

It had previously been assumed that FFA uptake by the muscle occurred by the process of passive diffusion as FFA are hydrophobic molecules and should be easily capable of diffusing through a hydrophobic plasma membrane (16, 33, 102). Recently however, a family of plasma membrane-bound proteins that bind to FFA have been identified (103, 104, 105, 106, 107). These proteins are recognized as plasma membrane fatty acid binding protein ($FABP_{PM}$), fatty acid translocase (FAT/CD36) and fatty acid transport protein (FATP) (16, 33). There also appears to be evidence that FFA

transport into the muscle may be chronically regulated. Conditions associated with an increased utilization of FFA, such as endurance training, increase the content of $FABP_{PM}$ (33). In addition, there is a greater content of $FABP_{PM}$ in slow twitch oxidative skeletal muscle than in fast twitch gylcolytic muscle (105, 108). It has also recently been established that FAT/CD36 can translocate from intracellular vesicles to the cell membrane, analogous to the redistribution of glucose transporter-4 (GLUT-4), indicating that FFA transport can also be acutely regulated (108, 109, 110). The improvement in fat oxidation following exercise has been partly attributed to the up regulation of FAT/CD36 (111). Holloway et al. (110) demonstrated an exercise induced increase in mitochondrial FAT/CD36 protein content which correlated with rates of palmitate oxidation. It appears increasingly likely that fatty acid transporters and binding proteins do exist in human skeletal muscle and that a large proportion of long chain fatty acids (LCFA) cross the membrane by facilitated transport rather than passive diffusion (11, 16, 33).

Free Fatty Acid Movement across the Mitochondrial Membrane

FFA are converted via fatty acyl-CoA synthetase into medium-chain fatty acids (MCFA) or LCFA (111). While MCFA can diffuse across the mitochondrial membrane, the LCFA are dependent on facilitated diffusion (Figure 3) via the carnitine palmitoyltransferase (CPT) complex for entry to the mitochondrion (111, 112). Upon entering the cell, LCFA are converted to long chain fatty acyl-CoA (LCFACoA) by acyl-CoA synthetase (ACS), a family of integral proteins present in various subcellular organelles, including the plasma membrane and the mitochondria (113, 114). The initial step in the transport of FFA into the mitochondria is the binding of carnitine with the acyl-CoA. This reaction is catalysed by the enzyme carnitine palmitoyltransferase 1 (CPT1) which spans the outer mitochondrial membrane. As carnitine binds with acyl-CoA, free

CoA is released. The resulting acylcarnitine complex is then transported through the inner mitochondrial membrane via carnitine-acylcarnitine translocase in exchange for free carnitine (115). The acylcarnitine is then reconverted into acyl-CoA at the matrix side of the inner mitochondrial membrane by the enzyme carnitine palmitoyltransferase II (CPTII) (115). The carnitine that is released diffuses back across the mitochondrial membrane into the cytoplasm and becomes available again for the transport of other FFA (16).

Pathway of Free Fatty Acid Oxidation

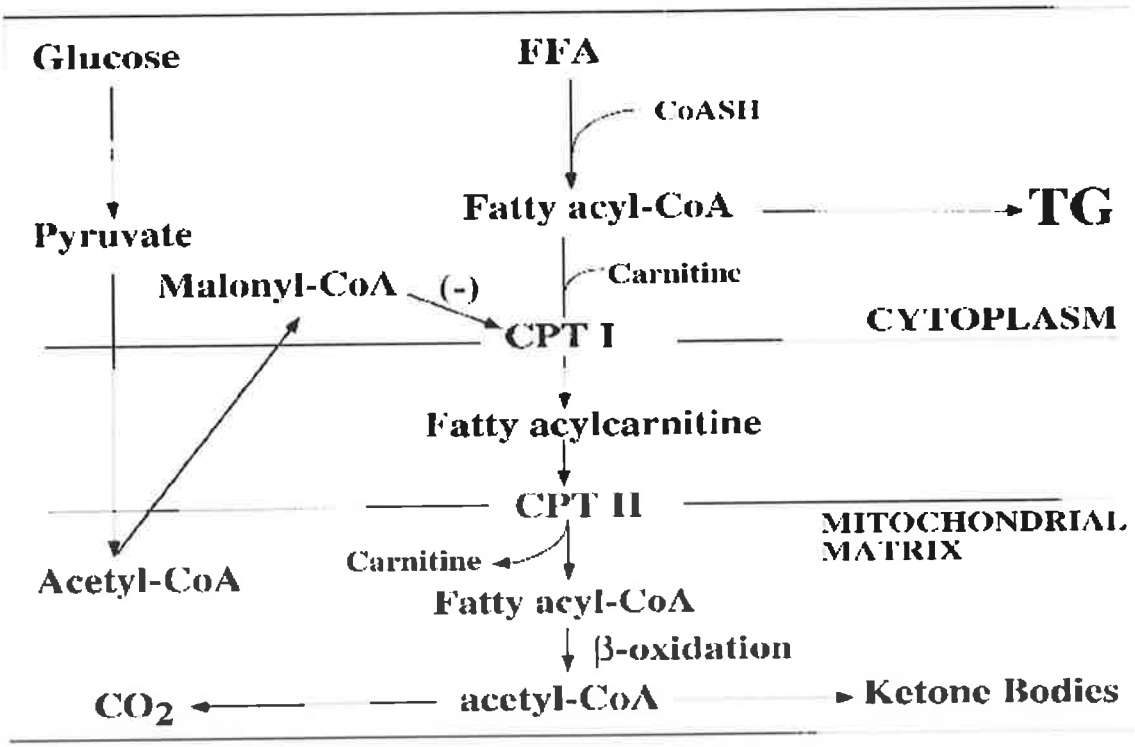


Figure 3: Schematic diagram showing the regulation of long-chain fatty acid entry into the mitochondria by the CPT complex and the impact of carbohydrate availability on CPT I activity. (adapted from Sidossis et al., 1996 (56))

CPTI is generally regarded as the rate limiting step in the transport of FFA across the mitochondria and may even be the rate limiting enzyme for fat oxidation (16). At least two isoforms of CPTI are expressed; L-CPTI a liver isoform and M-CPTI a smaller isoform expressed in cardiac and skeletal muscle (70, 116). A number of proposed regulators of CPTI activity have been highlighted, including malonyl CoA, hydrogen ion accumulation in the sarcoplasm and reduced free carnitine availability (16).

The Role of Malonyl-CoA and its Regulation by Acetyl-CoA Carboxylase and AMPK

Malonyl CoA is an intermediate of FFA synthesis and has been shown to be a potent inhibitor of CPTI activity at rest (70, 117, 118, 119). It is thought that the primary role of malonyl CoA in skeletal muscle is to regulate the rate of fat oxidation (120). Malonyl CoA is formed from acetyl-CoA, a reaction catalysed by the enzyme ACC. ACC exists in two isoforms, ACCα and ACCβ. ACCβ is the dominant isoform expressed in skeletal muscle and also appears to be associated with the outer mitochondrial membrane, possibly in close proximity to CPTI (70, 121). ACC is phosphorylated and inactivated by AMPK and allosterically by changes in the cytosolic concentration of citrate (69, 70). Exercise results in the activation of AMPK in muscle (Figure 4), which leads to the inactivation of ACC and consequent decline in malonyl CoA levels, releasing the inhibition on fat oxidation (122). It is also thought that AMPK phosphorylates and activates malonyl CoA decarboxylase (MCD) which helps lower malonyl CoA levels (70, 120, 122, 123, 124). Winder et al. (125) reported a decrease in malonyl-CoA levels in rodent skeletal muscle from rest to moderate intensity exercise, in conjunction with increasing fat oxidation. It is well established that acetyl-CoA levels in the muscle increase rapidly at the onset of high intensity exercise, thus stimulating the activity of ACC and subsequently increasing the concentration of malonyl CoA. This may help to explain the reduction in FFA uptake into the mitochondria during high intensity exercise

(120).

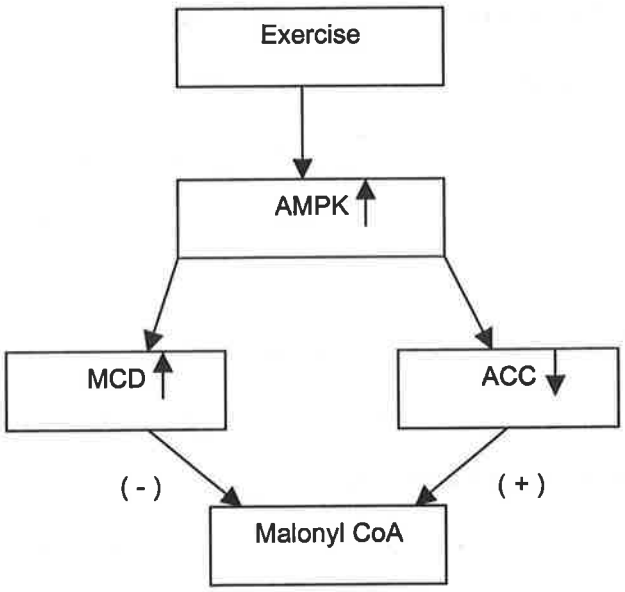


Figure 4: Coordinated changes in enzymes of lipid metabolism mediated through the exercise induced activation of AMPK.

It is hypothesized that the role of AMPK is to protect the cell against ATP depletion by inhibiting energy storing pathways and stimulating energy generating pathways (126). It is therefore in keeping that AMPK should inhibit ACC and malonyl CoA production while promoting MCD activity. Increases in AMPK activity in rat skeletal muscle following exercise (Figure 4) have been identified and are associated with significant increases in MCD activity and decreases in ACC activity and malonyl CoA concentration (123, 127). Rasmussen et al. (127) demonstrated a decrease in malonyl CoA levels and ACC activity in rat skeletal muscle for at least 90 min following 30 min of submaximal running.

It has been suggested that fuel availability also regulates AMPK activity (128). Glucose ingestion increases the concentration of malonyl CoA by decreasing the activity of AMPK and by increasing the cytosolic concentration of citrate, an allosteric activator of ACC (70, 126, 129). Glycogen depletion decreases the malonyl CoA concentration by increasing citrate levels and AMPK activity (73, 128, 129, 130, 131).

Studies in humans however provide us with little evidence of the regulatory effects of malonyl CoA on CPTI, at least during exercise (132). Research in human subjects has failed to demonstrate decreases in malonyl CoA levels during prolonged submaximal exercise despite elevated fat oxidation (132, 133, 134). Research has also shown that resting malonyl CoA levels are theoretically high enough to completely inhibit CPTI, although physiologically that is not the case (16, 135). It has been proposed that two pools of malonyl CoA exist in skeletal muscle, one in the cytoplasm and the other in the mitochondria (135). The measurement of total muscle malonyl CoA concentration would therefore inaccurately reflect the local malonyl CoA concentrations in close proximity to CPTI (135). This possible compartmentalization of malonyl CoA may complicate interpretation of measurements in whole tissue (102, 110). ACCβ has been demonstrated to be closely associated with the mitochondria; leading to speculation that malonyl CoA produced by ACCβ is synthesized in close proximity to CPTI, thus allowing inhibition of the enzyme without marked changes in total cellular malonyl CoA (136). It is possible that a significant decline in malonyl CoA concentration could occur adjacent to CPTI without any noticeable changes in total tissue malonyl CoA (132). It is apparent that the regulation of CPTI activity in human skeletal muscle is more complicated than regulation by malonyl CoA concentration alone (16, 131).

The Role of Hydrogen Ions

Hydrogen ion accumulation has also been proposed to have a potential regulatory effect on FFA uptake into the mitochondria. In rodent muscle the sensitivity of CPTI to malonyl CoA is pH dependent (16, 115, 137). At pH 6.8, CPTI binds to malonyl CoA more efficiently than at a more neutral pH, which is present in the muscle at rest or during low intensity exercise. As high intensity exercise is associated with increased muscle acidity, the reduction of fat oxidation at high exercise intensities could be the result of a pH-induced increased sensitivity of CPTI to malonyl CoA (15). In vitro studies performed in rat and human muscle have demonstrated reduced CPTI activity in response to very slight decreases in pH (138, 139). It is also likely that the activity of HSL is influenced by the acidic environment resulting from an accumulation of hydrogen ions. Hydrogen ion accumulation may therefore be partly responsible for the decrease in FFA and IMTG availability during, and in recovery from, high intensity exercise (16).

The Role of Carnitine

Carnitine is essential for mitochondrial β-oxidation (Figure 3) as it is used in the formation of long-chain acylcarnitine in a reaction with LCFACoA catalysed by CPTI (102). Carnitine may also be acetylated in a reaction with acetyl CoA catalysed by carnitine acetyl transferase (102). This results in carnitine acting as a sink for acetyl groups during conditions where the rate of acetyl CoA formation from pyruvate exceeds that from the TCA cycle (102, 131). Numerous studies have described a decreased availability of free carnitine with increasing exercise intensities, when glycolytic flux and acetyl-CoA formation are high (18, 136, 140, 141).

During low intensity exercise, the flux through pyruvate dehydrogenase (PDH) is less than the flux through the TCA cycle, the outcome being minimal acetylation of the

carnitine pool. However, as exercise intensity increases, the flux through PDH begins to increase more rapidly than the flux through the TCA cycle, resulting in an accumulation of acetyl-CoA. These acetyl-CoA molecules are bound to free carnitine to allow the release of CoA. This acetylation of the carnitine pool leads to a decrease in the concentration of free carnitine possibly reducing the transport of FFA into the mitochondria (18, 142). van Loon et al. (18) reported a shift from mainly free carnitine to acetylcarnitine with increasing exercise intensity.

It may be speculated therefore that the decreased concentration of free carnitine observed during high intensity exercise may limit CPTI activity, providing a situation whereby an increased availability of pyruvate down-regulates lipid metabolism (131, 143). However, it could also be argued that the low levels of free carnitine may still be sufficient to facilitate FFA transport, considering that carnitine is recycled and not consumed during this process. It has also recently been shown that increased glycogen levels result in significantly lower free carnitine levels, in comparison to free carnitine levels in a glycogen depleted state (15, 131). This may help to explain the increased rate of fat oxidation following glycogen depleting exercise, an effect that has been shown to be blunted with CHO ingestion in recovery. Reduced CPTI activity is regarded as possibly the main candidate responsible for the down regulation of fat oxidation during high intensity exercise (15).

Glucose-Free Fatty Acid Interactions

The regulation of fat oxidation is influenced greatly by CHO. The factors that regulate the interplay between fat and CHO oxidation in skeletal muscle are insufficiently understood (30). It was originally thought that Randle's classic glucose-FFA cycle could explain the reciprocal relationship between CHO and lipid metabolism (144).

The Glucose-Free Fatty Acid Cycle

The theory behind the glucose-FFA cycle proposes that an increase in plasma FFA concentration will result in an increase in FFA uptake, which will subsequently lead to increased β-oxidation in the mitochondria (145). The increase in the concentration of acetyl-CoA, the product of β-oxidation, will then inhibit PDH, preventing the further breakdown of pyruvate to acetyl-CoA. Increased formation of acetyl-CoA from FFA would also increase muscle citrate levels which may potentially inhibit phosphofructokinase, causing a reduction in the rate of glycolysis. This in turn, will lead to an accumulation of glucose-6-phosphate in the muscle which will inhibit hexokinase activity, thus reducing muscle glucose uptake (16, 145, 146).

Recently however, muscle biopsy studies involving exercise in humans with elevated plasma FFA levels, demonstrated no increase in muscle citrate levels, raising doubts regarding the traditional glucose-FFA cycle (143). Odland et al. (147) investigated the effects of increased FFA availability on substrate metabolism during low and moderate intensity exercise, by infusing TG plus heparin. The elevation in FFA levels led to a 4-fold increase in FFA uptake with a 23% reduction in glycogenolysis. Glucose uptake however remained unaffected leading the authors to believe that the regulation took place at the level of glycogen phosphorylase or PDH. PDH is phosphorylated and deactivated by PDH kinase, which is in turn activated by high ATP, acetyl-CoA and nicotinamide adenine dinucleotide (NADH) levels at rest. Odland et al. (147) demonstrated similar acetyl-CoA and ATP levels with and without elevated FFA concentrations, suggesting that the effect of increased FFA availability on substrate selection was mediated through mechanisms other than those proposed by the glucose-FFA cycle.

The majority of evidence supporting the glucose-FFA cycle originates from isolated muscle experiments and in vitro studies, with relatively modest information regarding its existence in human skeletal muscle. Although there is some confirmation of its presence at rest and during low intensity exercise, the glucose-FFA cycle has failed in its attempt to explain substrate selection during exercise of moderate to high intensities (16, 30).

The Glucose–Free Fatty Acid Cycle Reversed

The glucose-FFA cycle has also been shown to operate in reverse, with an increased availability of CHO having a significant effect on the contribution of FFA to total energy expenditure and reesterification into TG at rest and during exercise (55, 56, 57, 144, 148). A glucose effect on FFA metabolism could be controlled by either regulation of lipolysis or control of fat oxidation. The reduction in lipid oxidation following glucose ingestion is due, in part, to the coordinated effects of decreased FFA availability secondary to decreased adipose tissue lipolysis and fat oxidation at the muscle (148). CHO ingestion raises blood glucose concentration which subsequently promotes insulin secretion (55, 149, 150, 151, 152). The anti-lipolytic effects of insulin results in a reduced rate of lipolysis, thus lowering plasma FFA levels, while stimulating glucose uptake into the muscle through increased translocation of GLUT-4 proteins to the cell surface. As mentioned previously, glucose ingestion also affects the rate at which FFA are reesterified into TG by providing α-glycerol phosphate, the backbone of TG (153).

Reduced entry of LCFA into the mitochondria has been demonstrated with CHO ingestion in endurance trained men (30). It is therefore possible that an accumulation of cytosolic LCFACoA may result in the allosteric inhibition of HSL and subsequent reduction in IMTG hydrolysis, decreasing the availability of FFA for β-oxidation (32, 68,

154). Watt et al. (32) reported HSL activity to decrease in the second hour of exercise, coinciding with an accumulation of LCFACoA. The accumulated LCFACoA are likely diverted towards esterification as oxidation is down regulated (6).

Effect of Glucose on Fat Oxidation

Increased CHO oxidation also inhibits fat oxidation by increasing the content of malonyl CoA in the muscle (70, 135, 144, 155). When glycolysis is augmented, malonyl CoA synthesis is enhanced, thereby reducing the activity of CPTI and inhibiting the uptake of FFA by the mitochondria (6). Coyle et al. (30) reported reduced long chain palmitate uptake into the mitochondria, with no reduction in medium chain octanate uptake. This supports an inhibitory effect of CHO ingestion on fat oxidation mediated through malonyl CoA and CPTI as palmitate is dependent on CPTI for transport into the mitochondria whereas octanate is not (30). It has also been demonstrated that the concentration of malonyl CoA in rat soleus muscle is increased 2-6 fold when incubated for 20 min with glucose and insulin (71). High glucose availability at rest has also been shown to elevate the concentration of cytosolic citrate (131). A close correlation has been reported between increased malonyl CoA levels and increases in the whole cell concentration of citrate, and also to a greater extent, the sum of the concentration of citrate and malate (130). As noted previously, ACC is allosterically activated by changes in the cytosolic concentration of citrate. Malate is a counter ion for citrate efflux from the mitochondria and it is possible therefore that a change in its concentration could reflect a redistribution of citrate from the mitochondria to the cytosol (69).

Triglyceride Synthesis during Recovery from Exercise and the Impact of AMPK

Upon entering cells, LCFACoA are trafficked towards either β-oxidation or esterification into TG (156). It is less clear how CHO ingestion and insulin increases FFA incorporation into TG (156). Following glycogen depleting exercise, fat oxidation is increased as available glucose is partitioned towards glycogen repletion. Subsequently, FFA esterification and storage as TG is reduced. With CHO ingestion during recovery from exercise, the metabolic response to the exercise-induced energy deficit is altered (22). Fat oxidation has been shown to be reduced and TG synthesis enhanced following glucose intake in the post exercise period (111, 153)

AMPK has been shown to regulate the partitioning of FFA into glycerolipid synthesis or β-oxidation (156). In addition to regulating the activity of various enzymes involved in the fat oxidation pathway, it has been suggested that AMPK may also inactivate enzymes involved in glycerolipid synthesis, thus inhibiting TG synthesis and promoting fat oxidation (156). mtGPAT has been shown to be regulated through phosphorylation by AMPK (156). Muoio et al. (156) studied liver and skeletal muscle lipid metabolism in isolated hepatocytes and soleus muscle from rats and mice. In both hepatocytes and isolated skeletal muscle, AICAR decreased [^{14}C]oleate incorporation into TG, DAG and phospholipids by 45-95%. In rat hepatocytes, AICAR resulted in the inhibition of mtGPAT activity, suggesting that the decreased TG synthesis seen with AICAR may be due to the AMPK-mediated inactivation of mtGPAT. In skeletal muscle, the specific activity of GPAT is very low and as a result the direct effects of AICAR were unable to be tested. However, the authors propose that the similar reduction in [^{14}C]oleate incorporation into TG seen in muscle as in liver implies that AICAR's effects on GPAT in both tissues are comparable (156). It may be argued therefore that the increase in fat oxidation and suppression of TG synthesis post exercise may be

mediated by the enhanced AMPK activation seen following exercise, resulting in the inactivation of ACC and the inhibition of GPAT (95). mtGPAT and CPTI are both positioned on the outer mitochondrial membrane and as a result, mtGPAT is in an ideal location to compete with CPTI for acyl-CoA's and influence their partitioning towards either β-oxidation or TG synthesis. AMPK possibly eliminates CPTI's competitor by inactivating mtGPAT, helping to reduce TG synthesis and partition FFA towards β-oxidation in the mitochondria (156, 157).

In perfused rat liver, cardiomyocytes and cultured adipocytes, insulin has been shown to acutely activate GPAT. However, the majority of these studies did not differentiate between the mitochondrial and endoplasmic reticulum isoforms of the enzyme (84). In a study by Muoio et al. (156) incubation of isolated mouse skeletal muscle with insulin resulted in a 15-fold increase in the esterification of [^{14}C]oleate into TG. When AICAR was added to the media, a 78% reduction in this ratio was recorded (156). It could be concluded that AMPK counteracts the action of insulin on FFA trafficking, possibly via their common targets of ACC and malonyl CoA and GPAT. This may explain the lack of a complete suppression of fat oxidation following glycogen depleting exercise, even in the presence of CHO ingestion (22). Horowitz et al. (22) demonstrated that in the presence of sufficient CHO, an exercise induced energy deficit increased plasma FFA concentration and suppressed CHO oxidation. van Loon et al. (158) reported impaired IMTG repletion during recovery from exercise with a CHO rich diet. This implies that in the post exercise recovery period, not all FFA are partitioned towards TG synthesis. Kiens and Richter (84) lend support to this hypothesis, demonstrating a continued reduction in IMTG concentrations during recovery from exercise, despite a large CHO intake, suggesting that substantial fat oxidation continues to occur in the presence of ample CHO supply. It is proposed that muscle glycogen

repletion is of such high metabolic priority following glycogen depleting exercise, that fat oxidation is necessary to meet energy demands (84, 85). As AMPK increases glucose transport and enhances glycogen synthesis, it can be presumed that it is also partly responsible for promoting muscle glycogen repletion following exercise (69). Park et al. (122) reported a 50% decrease in malonyl CoA levels and ACC activity with a concurrent 2-fold increase in MCD activity and increased AMPK activity in sedentary rats following 30 min of treadmill running. GPAT activity was also found to be diminished in liver and adipose tissue although no such effect was seen in skeletal muscle (122). The authors conclude that MCD, ACC and GPAT are coordinately regulated by AMPK in liver, adipose tissue and possibly in skeletal muscle following exercise and suggest the net effect of this regulation to be enhanced fat oxidation and diminished FFA esterification (122). Park et al. (122) demonstrated AMPK activity to be increased at 30 min post exercise, raising the possibility that the exercise induced increase in AMPK may play a role in regulating the long term effects of exercise on metabolism via signal transduction and gene expression.

It should also be noted that the content of glycogen in the muscle may exert a direct effect on metabolism following exercise (159). Restriction of CHO intake during recovery prevents the replenishment of muscle glycogen stores, raising the possibility that muscle glycogen content may be linked to the regulation of metabolism in the post exercise period (159). Pilegaard et al. (159) demonstrated prolonged activation of exercise-responsive metabolic genes such as CPTI and FAT/CD36 with low CHO feeding during recovery from 75 min of exercise at 75% $\dot{V}O_{2peak}$. A reversal in this activation was found with high CHO feeding (159).

Conclusions

It is clear that a number of factors influence substrate metabolism during and after exercise. That an increase in lipid oxidation occurs in the post exercise period is well supported (11, 12, 13, 14). One possible explanation for this is that following exercise, glycogen repletion is of high metabolic priority. CHO is therefore channeled towards replenishing muscle glycogen stores and lipids used for energy supply (23, 24, 25). The mechanisms responsible for the increase in fat oxidation during recovery remain incompletely understood. It is thought that AMPK actively partitions FFA away from reesterification and towards β-oxidation by exerting an effect on ACC, MCD and GPAT (122). CHO ingestion alters substrate metabolism by decreasing the rate of fat oxidation and increasing esterification rates, primarily via an increase in insulin and the subsequent effects on lipolysis (55, 56, 144, 148). There is some evidence to suggest that during recovery from exercise, the effect of a rise in insulin levels resulting from CHO ingestion may be overridden by the concurrent increase in AMPK activity. This helps to explain how lipid oxidation has been reported to remain high following exercise, even in the face of substantial CHO ingestion (22).

Chapter 3: Methodology

Subjects

Eight young, healthy, moderately trained males volunteered to participate in the study. Subjects were recruited from the staff and student population at Dublin City University (DCU). Subjects were fully informed of the nature and possible risks of the study and provided written consent in accordance with Dublin City University Institutional Review Board. All subjects were non smokers with a normal body mass index. Subjects were excluded if they did not exercise regularly, had diabetes or used drugs that the study physician and investigators decided would interfere with the normal adaptation to the proposed intervention.

Preexperimental Testing

Subjects initially completed a health history questionnaire and were examined by the study physician to determine eligibility. Lange skinfold callipers (Cambridge Scientific Industries, MD) were used to measure double thickness subcutaneous adipose tissue and the equation by Brozak et al. (160) was used to calculate percent body fat.

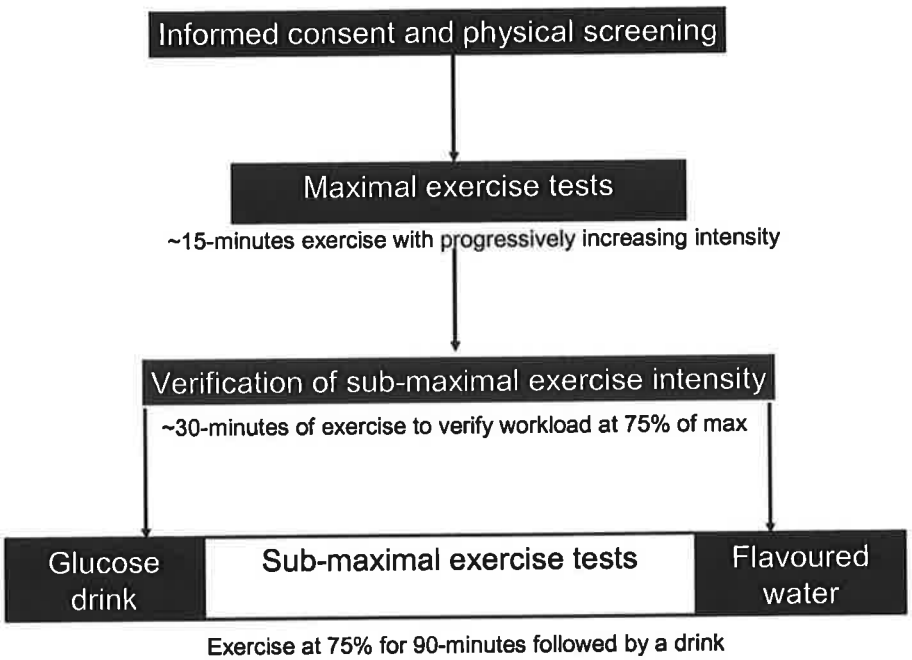
Subjects then performed an incremental exercise test on a bicycle ergometer to determine $\dot{V}O_{2peak}$. Subjects warmed up at 100 watts (W) for 5 min at a self-selected cadence between 60 and 80 revolutions per minute (rpm). Following the warm-up, subjects maintained their selected cadence and exercise intensity was increased in increments of 50 W every 2 min until the subject reached volitional exhaustion, at which point the test was terminated. The test was deemed maximal if 3 or more of the following criteria were satisfied; volitional exhaustion, leveling of oxygen consumption (<0.2 l·min^{-1}), RER > 1.1 and heart rate within 10 beats of the subjects' age predicted maximum heart rate. Respiratory measurements were determined using a

sensormedics Vmax 229 metabolic cart (Sensormedics Corp., Yorba Linda CA). Ratings of perceived exertion (RPE) were obtained throughout the test using the 15-point Borg category RPE scale (161). Heart rate was continuously monitored during exercise using a wireless Polar heart rate monitor (Polar Vantage NV™ Polar, Port Washington, NY).

No sooner than 4 days following the maximal exercise test, subjects were required to perform a submaximal exercise session to determine the workload corresponding to 75%$\dot{V}O_{2peak}$. The workload was deemed to correspond to 75%$\dot{V}O_{2peak}$ providing oxygen uptake remained in a steady state for a minimum of 10 min at that intensity.

Experimental Design

Subjects underwent two experimental trials separated by 1-2 weeks. Each trial consisted of a 90 min bout of exercise on a bicycle ergometer at 75%$\dot{V}O_{2peak}$. In one trial, subjects consumed a carbohydrate drink containing 1.5 g glucose per kg body weight in a 10% solution (CHO), and in the other, a flavoured placebo (PLA) similar in volume, immediately upon completion of exercise. The order of the CHO and PLA trials was randomly assigned.



Dietary Control

On the 3 days preceding the first experimental trial, subjects recorded their habitual dietary intake by a self-reported food diary. On the 3 days preceding the second experimental trial, subjects followed a diet similar to the one ingested before the first trial.

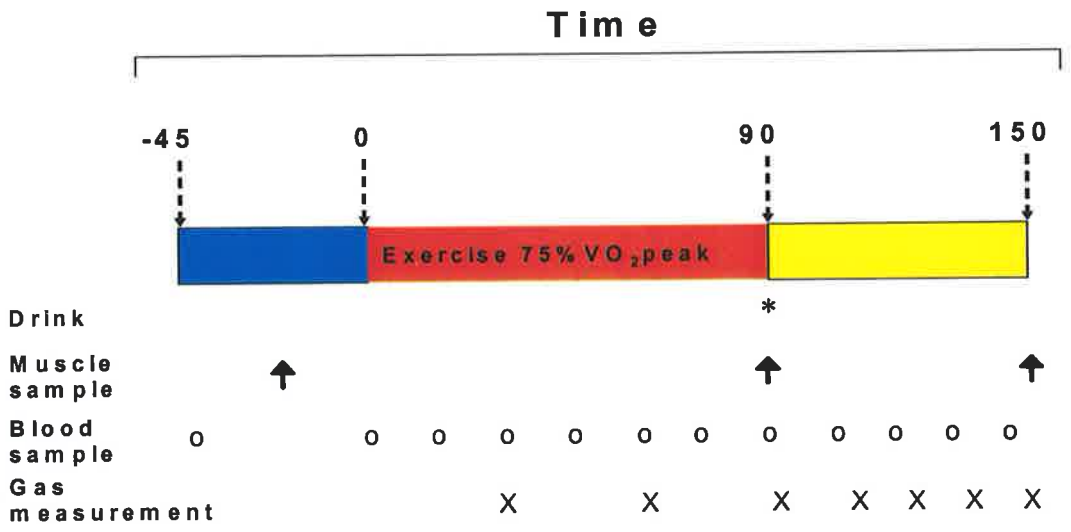
Experimental Trials

Subjects arrived at the Metabolic Research Unit in the School of Health and Human Performance at DCU on the morning of the experimental trials. All subjects were required to refrain from any heavy physical activity for 24 h before each trial and to abstain from alcohol and caffeine ingestion for 48 h before each trial. After 30 min of rest in a supine position, a muscle biopsy was obtained under local anesthesia from the vastus lateralis muscle by the needle biopsy technique. Subjects then had a 21 G indwelling catheter inserted into a prominent vein in the forearm to facilitate the

collection of blood samples during the recovery period.

Following the collection of a baseline blood sample, subjects initiated exercise. Exercise consisted of 90 min of bicycle ergometry at a workload corresponding to approximately 75% $\dot{V}O_{2peak}$. Cadence was maintained between 60-80 rpm throughout. Expired gases were analysed for the first 15 min of exercise, between 35-45 min and again between 75-85 min by means of a mouthpiece connected to a sensormedics Vmax 229 metabolic cart, to verify exercise intensity. Approximately 20 min before the end of exercise, subjects briefly paused cycling while a local anesthetic was administered into the vastus lateralis. Exercise was continued within 120 s. Immediately at the end of exercise, a muscle biopsy was obtained from the anaesthetized area and a blood sample taken from the indwelling catheter. Subjects then ingested either a CHO drink or PLA. On completion of the drink, subjects rested in a supine position for the remainder of the hour. Substrate oxidation and energy expenditure were recorded during this time by means of a ventilated canopy. Blood samples were taken every 15 min from the end of exercise. A third muscle biopsy was taken 1 h after exercise.

Heart rate was monitored throughout the exercise trial with a wireless Polar heart rate monitor and RPE was obtained every 10 min during exercise. During the first experimental trial, subjects were offered water ad libitum, and water intake recorded. During the second experimental trial, subjects repeated their water intake from the first trial. Subjects were permitted to listen to music; however, if music was not used during the first trial, it was not permitted during the second. (See Appendix A for data collection sheets).



Breath and Blood Samples

Energy expenditure and fat and CHO oxidation rates were calculated during exercise and recovery using the thermal equivalents for oxygen consumption at different non-protein RQ values (162).

Glucose and lactate samples were placed directly on ice and analysed as soon as possible. Serum samples were left to coagulate at room temperature for 30 min before being placed on ice. Blood glucose and lactate concentrations were measured using an automated YSI 2300 Glucose Analyser. Spectrophotometric analyses were performed on an automated bench-top clinical chemistry system (ACE®, Alfa Wasserman B.V., Netherlands) for determination of serum triglyceride and free fatty acids using appropriate reagents, calibrators and controls (Randox Laboratories, UK). Intra-assay co-efficients of variation were <3% in all cases. Concentrations of insulin in the blood were determined by time-resolved fluoroimmunoassay (1235 AutoDELFIA automatic immunoassay system).

Muscle Biopsies

Muscle biopsies were quick frozen (< 20 s after being obtained) in liquid nitrogen and stored at -80^{O}C for subsequent biochemical analysis. Approximately 60 milligrams wet weight was freeze dried and dissected free of all visible adipose tissue, connective tissue and blood under a microscope. All biopsies were obtained through separate incisions spaced at least 3 cm apart. Muscle glycogen concentration was determined on 2 mg dry weight while the remaining dry weight was used for protein analysis.

Muscle Glycogen

2 mg freeze dried muscle was allowed thaw to -15^{0}C. Muscle was incubated in 0.5 ml of 2N hydrochloric acid for 2 h at 100^{0}C. Samples were then reconstituted to original weight with dH_2O before being neutralised with 1.5 ml of 0.67N NaOH. 1 ml of reagent mix containing Tris base, HCl, $MgCL_2$, DTT, ATP, NADP, HK and G-6-PDH was added to samples and glycogen content determined by fluorometer.

Western Blotting

Muscle was homogenised in a buffer containing 1.5 M NaCl, 200 mM KCl, 200 mM $MgCl_2$, 100 mM Na_3VO_4, 20% Triton X-100, Glycerol, 1 M Tris pH 8.0, 200 mM PMSF, 1 mM NaF, 1mM EDTA and protease inhibitor. Homogenates were centrifuged and the supernatant removed and frozen. Protein concentration of the muscle lysates was subsequently determined using the DC protein assay based on the Lowry method (163). Muscle lysates were solubilised in Laemmli sample buffer and boiled for 5 min before being separated by SDS-PAGE analysis on 10% acrylamide gels. Proteins were then transferred to a nitrocellulose membrane and blocked for 2 h in 3% BSA in 1 x Tris Buffer Saline with 0.02% Tween (TBS-t). Membranes were then sectioned and incubated in primary antibody overnight. The next day, membranes were washed in

TBS-t for 1 h and then incubated for 2 h in secondary antibody before being washed again. Primary antibodies Phospho-Acetyl-CoA Carboxylase [Ser79] (#3661 Cell Signaling), Phospho-AMPKα [Thr172] (#2531 Cell Signaling) and Phospho-HSL [Ser565] (#4137 Cell Signaling) and secondary antibody Goat Anti-Rabbit IgG, HRP-conjugate (12-348 Upstate) were used. Membranes were developed with enhanced chemiluminescence (SuperSignal West Pico Luminol/Enhancer Solution, Pierce) and quantified by densitometry (Image J, NIH). Phosphorylation of ACC, AMPK and HSL was measured as band density relative to a standard sample which was run on all blots.

Statistics

SPSS (version 12.0.1 for Windows, SPSS Inc.) statistical software was used to perform the statistical analysis. Data are presented as mean ± standard error (SE). Statistical significance was accepted at the $p < 0.05$ level of confidence, unless otherwise stated. Descriptive statistics were calculated for the eight subjects. Paired t-tests were used to compare energy expenditure and carbohydrate and fat oxidation between trials and to test for differences in variables independent of time. For variables measured before and after exercise a repeated measures ANOVA was performed to test for differences between protocols or changes due to time. When a significant main effect of time was found, significant pairwise differences were performed using Tukey's post hoc test.

Chapter 4: Results

Whole-Body Responses

A summary of physical and physiological characteristics of the subjects is presented in Table 1. Subjects were lean, healthy and moderately trained.

Table 1: Subject Physical Characteristics

Characteristic	
Age (yr)	23.4 ± 1.7
Height (cm)	184.2 ± 2.4
Weight (kg)	78.8 ± 2.8
BMI ($kg \cdot m^{-2}$)	23.2 ± 0.5
Fat Mass (kg)	7.6 ± 1.2
Lean Body Mass (kg)	71.2 ± 1.8
Percent Body Fat (%)	9.4 ± 1.1
$\dot{V}O_{2peak}$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	58.0 ± 2.1
$\dot{V}O_{2peak}$ ($l \cdot min^{-1}$)	4.6 ± 0.1

Data are presented as means ± SE

Oxygen Consumption and Energy Expenditure during Exercise

The percent of $\dot{V}O_{2peak}$ at which subjects exercised was not different between trials (71.5 ± 1.8% $\dot{V}O_{2peak}$ and 72.5 ± 1.8% $\dot{V}O_{2peak}$ for CHO and PLA trials respectively, $p > 0.05$) and there was no difference in the rate (16.0 ± 0.4kcals·min^{-1} and 16.2 ± 0.4 kcals·min^{-1} for CHO and PLA trials respectively, $p > 0.05$) or total amount (1439 ± 33 kcals and 1459 ± 39 kcals for CHO and PLA trials respectively, $p > 0.05$) of energy expenditure (Table 2).

Table 2: Oxygen Consumption and Energy Expenditure during Submaximal Exercise

	CHO	PLA	P value
$\dot{V}O_2$ (ml·kg^{-1}·min^{-1})	41.4 ± 1.6	41.9 ± 1.3	.457
Percent $\dot{V}O_{2peak}$ (%)	71.5 ± 1.8	72.5 ± 1.8	.420
Energy Expenditure (kcals·min^{-1})	16.0 ± 0.4	16.2 ± 0.4	.446
Total Energy Expenditure (kcals)	1439 ± 33	1459 ± 39	.454

Data are presented as means ± SE

Post Exercise Substrate Oxidation

Substrate oxidation rates were assessed for 1 h following exercise. The steady state data from the final 10 min of the hour was used to determine post exercise energy expenditure (kcals·min^{-1}) and the rate of fat and carbohydrate oxidation. The rate of energy expenditure (kcals·min^{-1}) following exercise (Figure 5) was not different between trials (1.95 $\pm$ 0.07 kcals·min^{-1} and 1.84 $\pm$ 0.11 kcals·min^{-1} for CHO and PLA trials respectively, $p > 0.05$). Post exercise RQ (Figure 6) was higher in the CHO trial than in the PLA trial (0.87 $\pm$ 0.02 and 0.82 $\pm$ 0.02 respectively, $p < 0.05$).

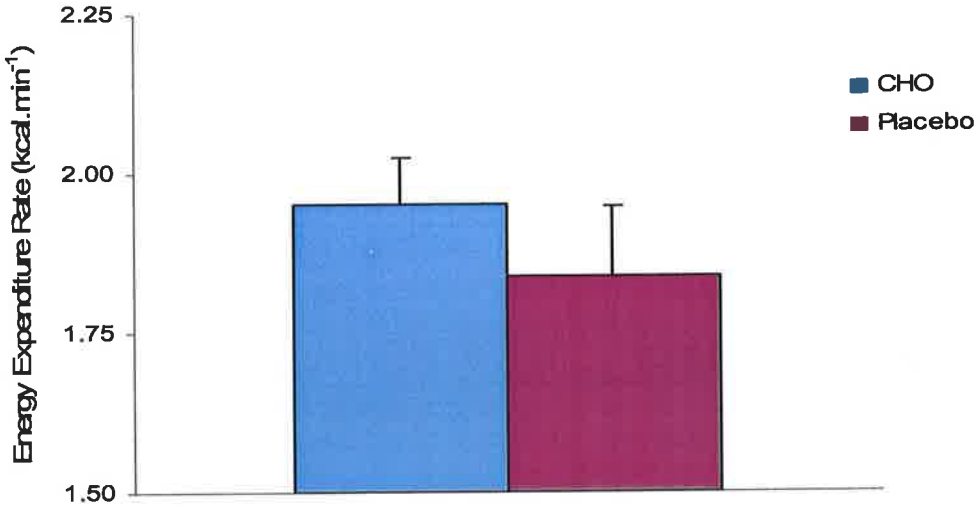


Figure 5: The Rate of Whole Body Energy Expenditure ($kcal \cdot min^{-1}$) Measured 1 h Post Exercise.

Data presented as mean ± SE. Significance set at $p<0.05$

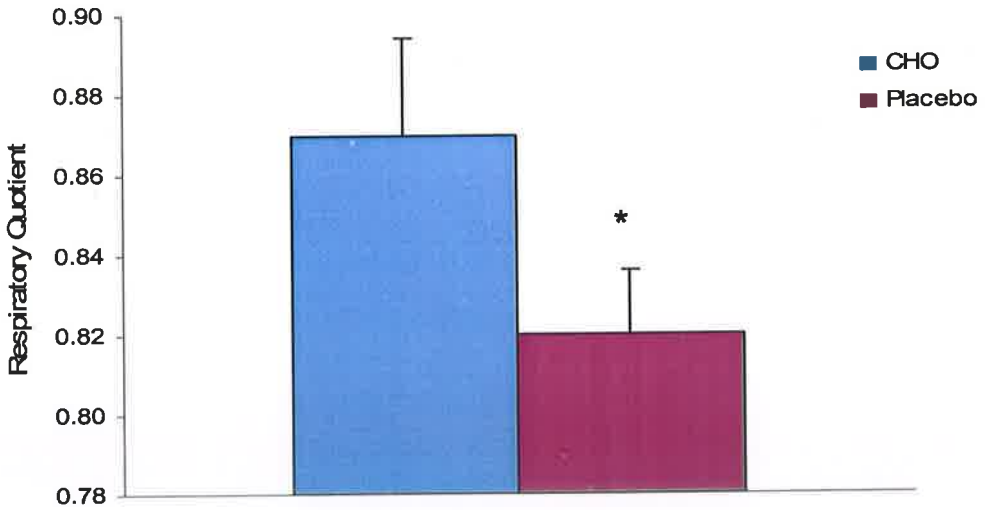


Figure 6: The Non-Protein Respiratory Quotient Measured 1 h Following Exercise in Carbohydrate and Placebo Trials.

Data presented as mean ± SE. * Significantly different from CHO trial ($p < 0.05$)

A summary of post exercise carbohydrate and fat oxidation is outlined in Figure 7. The rate of carbohydrate oxidation ($g \cdot min^{-1}$) was significantly greater in the CHO trial 1 h post exercise (0.25 $\pm$ 0.03 and 0.16 $\pm$ 0.02 $g \cdot min^{-1}$ for the CHO and PLA trials, respectively, $p < 0.05$). The rate of fat oxidation ($g \cdot min^{-1}$) was not significantly different between trials at this time point (0.10 $\pm$ 0.02 vs. 0.12+0.02 $g \cdot min^{-1}$ for the CHO and PLA trials, respectively, $p>0.05$). There was no difference between fat and carbohydrate oxidation (0.12 $\pm$ 0.02 and 0.16 $\pm$ 0.02 $g \cdot min^{-1}$ respectively, $p > 0.05$) following exercise in the PLA trial. The contribution of fat towards energy expenditure (%EE) during recovery from exercise was lower in the CHO trial (44.7 $\pm$ 7.4%EE) than in the PLA trial (61.0 $\pm$ 5.5%EE, $p < 0.05$). The opposite was true for the contribution of carbohydrate (55.3 $\pm$ 7.4%EE and 39.0 $\pm$ 5.5%EE for CHO and PLA trials respectively, $p > 0.05$, Figure 8).

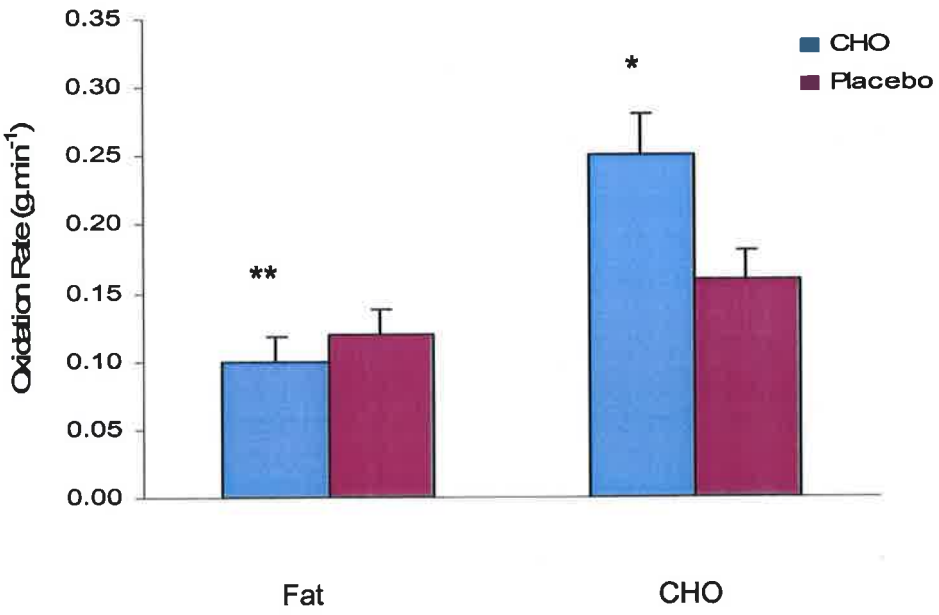


Figure 7: The Rates of Carbohydrate and Fat Oxidation 1 h Post Exercise for the Carbohydrate and Placebo Trials.

Data are presented as mean ± SE. * CHO oxidation significantly different from PLA trial ($p < 0.05$); ** Fat oxidation significantly different from CHO oxidation, CHO trial ($p < 0.05$)

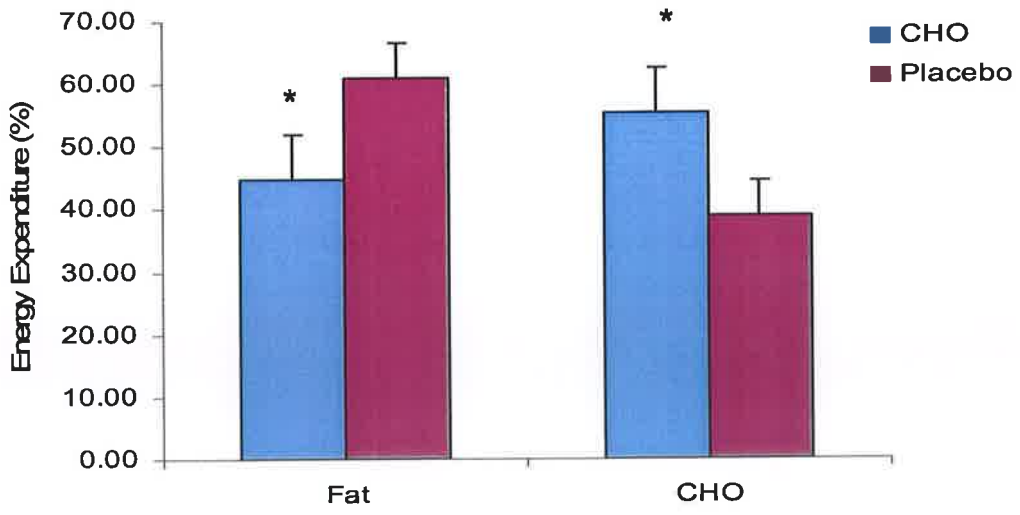


Figure 8: The Relative Contribution of Carbohydrate and Fat Oxidation to Total Energy Expenditure in the 1 h Post Exercise for the Carbohydrate and Placebo Trials.

Data are presented as mean ± SE. * Significantly different from PLA trial ($p < 0.05$)

Substrate Availability

Plasma Glucose

A summary of the plasma glucose response during exercise and recovery is outlined in table 3. Exercise had no effect on blood glucose levels in either trial. At 30 and 60 min post exercise, plasma glucose was significantly lower than baseline in the PLA trial (5.01 $\pm$ 0.09mmol·l^{-1}, 4.60 $\pm$ 0.13 and 4.41 $\pm$ 0.14 for baseline, 30 min post and 60 min post, respectively, $p < 0.001$). In the CHO trial, blood glucose levels were increased significantly above baseline at all time points after CHO ingestion (Table 3). Blood glucose was significantly higher in the CHO trial than the PLA trial at all time points from 30 min post exercise ($p < 0.05$).

Table 3: The Plasma Glucose Responses (mmol·l^{-1}) to Exercise and Recovery in the Carbohydrate and Placebo Trials

	CHO	Placebo	P value
Baseline	4.79 ± 0.19	5.01 ± 0.09	.286
End of Exercise	4.62 ± 0.22	4.58 ± 0.18	.815
15 min Post Exercise	6.18 ± 0.51*	5.92 ± 0.81	.788
30 min Post Exercise	8.47 ± 0.69*	4.60 ± 0.13*	.000
45 min Post Exercise	8.34 ± 0.49*	4.50 ± 0.14*	.000
60 min Post Exercise	7.57 ± 0.53*	4.41 ± 0.14*	.000

Data are presented as means ± SE. The P value represents comparisons between trials.
* Significantly different from baseline

Serum Insulin

The serum insulin response to exercise and recovery are presented in Figure 7. A tabular list of the data is presented in Appendix B. Exercise resulted in a significant drop in serum insulin levels from 4.75 ± 0.75 $\mu U \cdot mL^{-1}$ to 1.47 ± 0.57 $\mu U \cdot mL^{-1}$ in the CHO trial and from 3.63 ± 0.44 $\mu U \cdot mL^{-1}$ to 1.44 ± 0.70 $\mu U \cdot mL^{-1}$ in the PLA trial ($p < 0.05$). In the 1 h following exercise in the PLA trial, the serum insulin concentrations were similar to baseline ($p > 0.05$). In the CHO trial, insulin concentrations were significantly greater than baseline at every time point from 15 min post exercise ($p < 0.05$). At 15 min post exercise and every subsequent time point, serum insulin levels were greater following CHO ingestion than following PLA ingestion ($p < 0.05$).

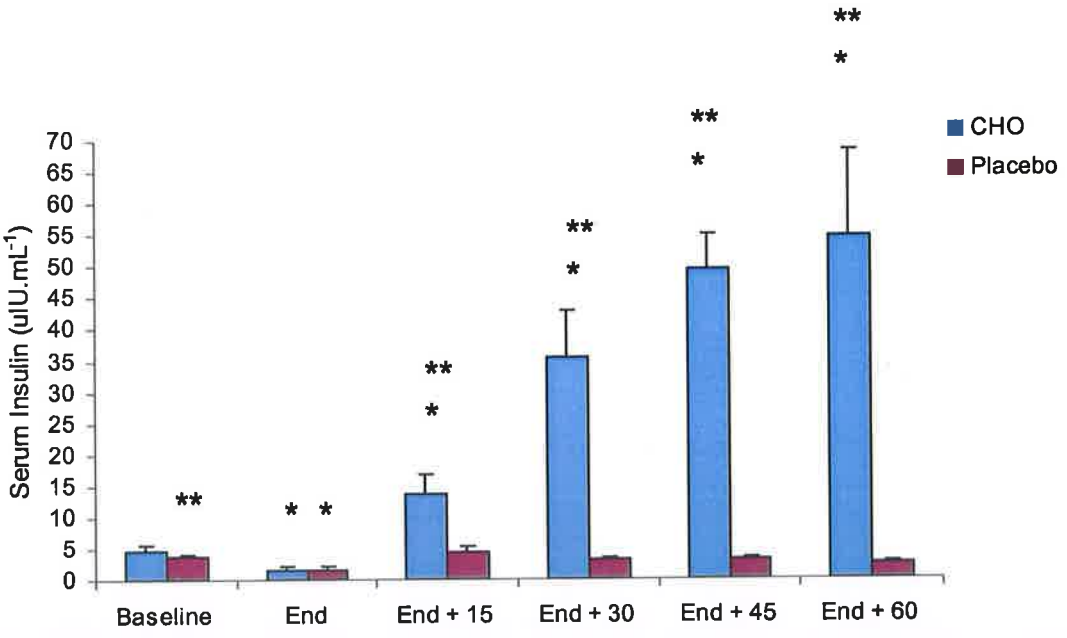


Figure 9: The Serum Insulin Response to Exercise and Recovery in the Carbohydrate and Placebo Trials.

Data are presented as mean ± SE. * Significantly different from baseline. ** Significantly different from PLA trial

Non-Esterified Fatty Acids

The non-esterified fatty acid responses to exercise and recovery are presented in Figure 8. A tabular list of the data is presented in Appendix B. NEFA were similar at baseline for both the CHO and the PLA trials (0.81 $\pm$ 0.10 and 0.70 $\pm$ 0.07 $mmol \cdot l^{-1}$ respectively, $p > 0.05$). In both the PLA and CHO trials, NEFA were higher than baseline immediately following exercise and reached their peak levels (1.89 $\pm$ 0.28 $mmol \cdot l^{-1}$ and 2.17 $\pm$ 0.27 $mmol \cdot l^{-1}$ in the CHO and PLA trials respectively) at 15 min recovery ($p < 0.05$). At 30 min, 45 min and 60 min post exercise in the PLA trial, NEFA were lower than at 15 min post but remained significantly higher than baseline ($p < 0.05$). In the CHO trial, NEFA remained above baseline at 30 min post exercise, before decreasing to below baseline level at 60 min post ($p < 0.05$). At 60 min recovery, NEFA were higher in the PLA trial (1.36 $\pm$ 0.18 $mmol \cdot l^{-1}$) than in the CHO trial (0.43 $\pm$ 0.06 $mmol \cdot l^{-1}$, $p < 0.05$).

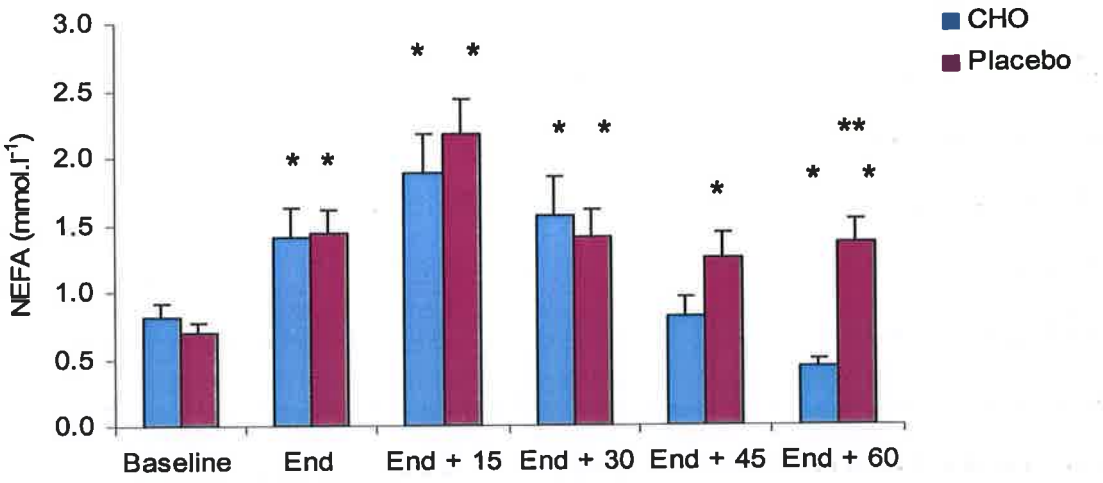


Figure 10: The Non-Esterified Fatty Acid Responses to Exercise and Recovery in the Carbohydrate and Placebo Trials.

Data are presented as mean ± SE. * Significantly different from baseline ($p<0.05$). ** Significantly different from CHO trial

Muscle Glycogen

There was no difference in muscle glycogen levels at any time point between trials ($p > 0.05$). Muscle glycogen was significantly lower ($p < 0.05$) following exercise in both trials (23.7% and 24.3% of baseline in CHO and PLA trials respectively). Glycogen levels were higher at 1 h recovery than at the end of exercise although not significantly so, despite carbohydrate ingestion in the CHO trial ($p < 0.05$, Figure 11). A tabular list of the data is presented in Appendix B.

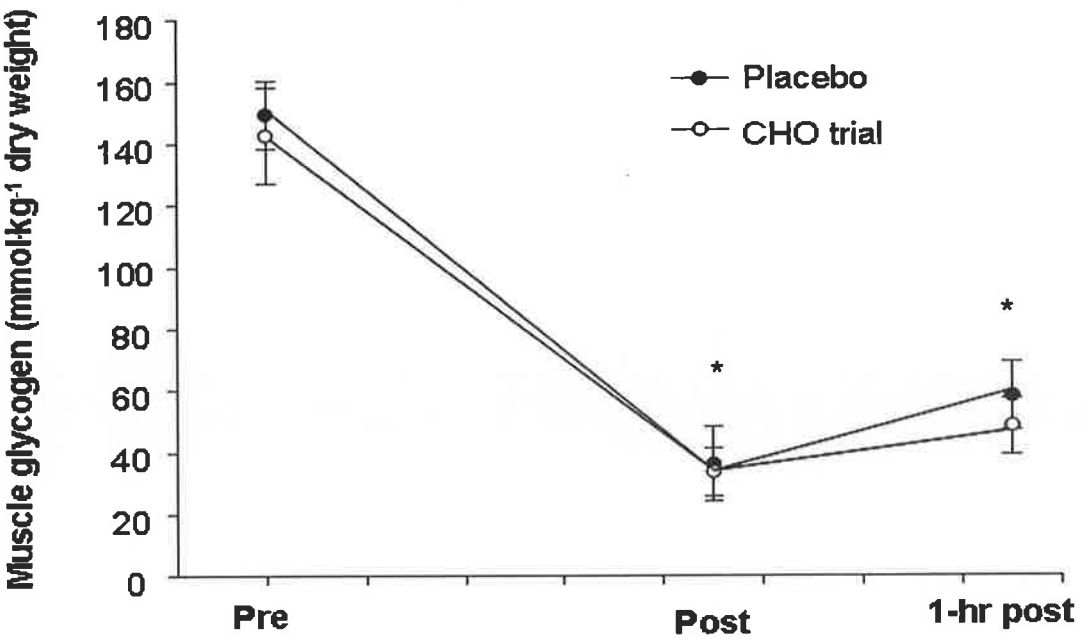


Figure 11: Muscle Glycogen Concentrations in Response to Exercise and Recovery for the Carbohydrate and Placebo Trials.

Data are presented as mean ± SE. * Significantly lower than baseline for CHO and PLA trials ($p<0.05$)

Phosphorylation of Acetyl-CoA Carboxylase, AMPK and Hormone Sensitive Lipase

Acetyl-CoA Carboxylase Phosphorylation

Phosphorylation of ACC in muscle was measured before and after exercise and after 1 h recovery. ACC phosphorylation was increased similarly in both trials immediately after exercise ($p < 0.05$). CHO ingestion resulted in a significantly lower ACC phosphorylation compared with PLA ingestion ($p < 0.05$, Figure 12). A tabular list of the data is presented in Appendix B

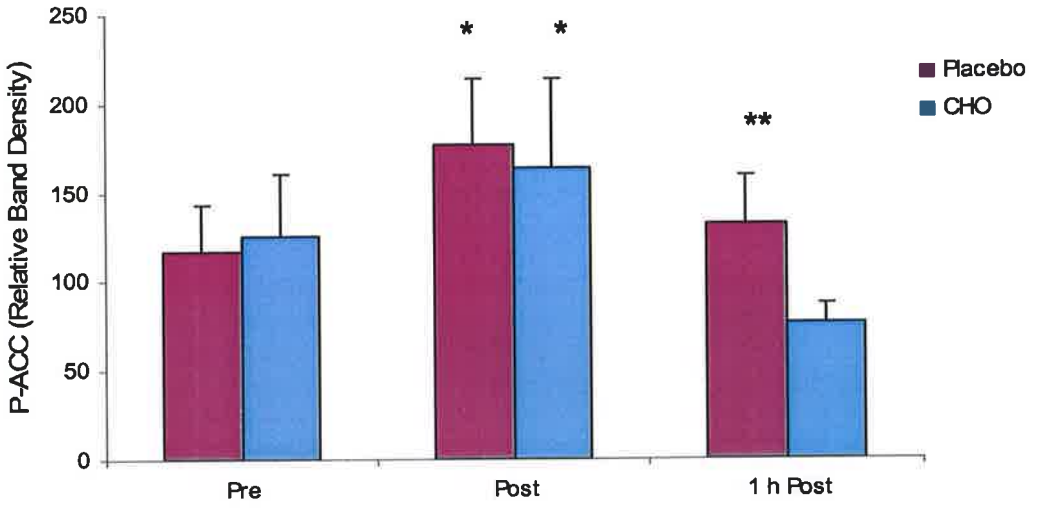


Figure 12: Acetyl-CoA Carboxylase Phosphorylation in Response to Exercise and Recovery for the Carbohydrate and Placebo Trials.

Data are presented as mean ± SE. * Significantly different from baseline for PLA and CHO trials. ** Significantly different from CHO trial

AMPK Phosphorylation

AMPK phosphorylation (Figure 13) in the muscle was similar between trials before exercise and increased similarly immediately after exercise. AMPK phosphorylation at 1 h post exercise was lower in the CHO trial than in the PLA trial. A tabular list of the data is presented in Appendix B

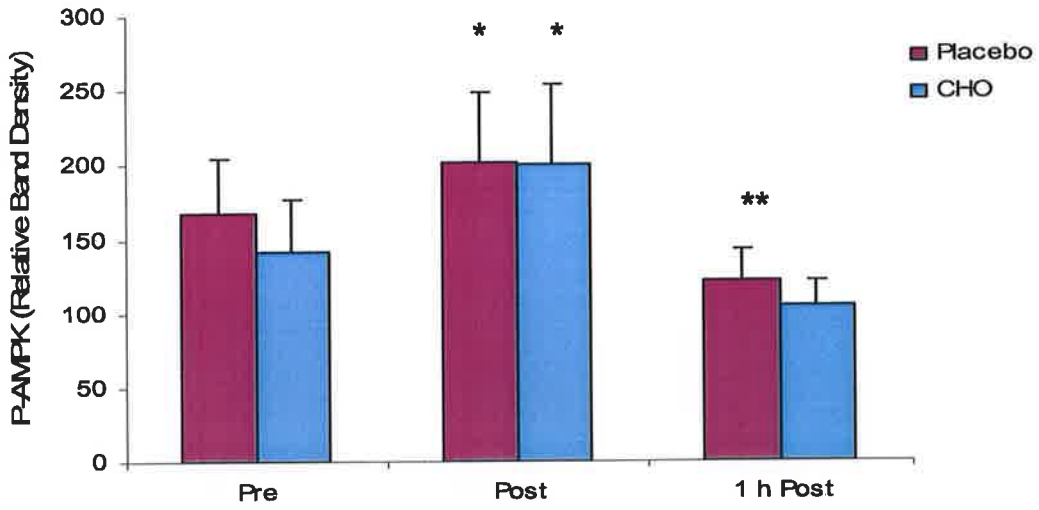


Figure 13: Muscle AMPK Phosphorylation in Response to Exercise and Recovery for the Carbohydrate and Placebo Trials.

Data are presented as mean ± SE. * Significantly different from baseline. ** Significantly different from CHO trial

Hormone Sensitive Lipase Phosphorylation

Phosphorylation of HSL in the muscle was similar at baseline in both the CHO and the PLA trials ($p > 0.05$). In the PLA trial, phosphorylation increased with exercise and remained high throughout recovery ($p<0.05$). In the CHO trial, HSL phosphorylation was higher ($p < 0.05$) than baseline immediately following exercise but not at 1 h recovery ($p > 0.05$, Figure 14). A tabular list of the data is presented in Appendix B.

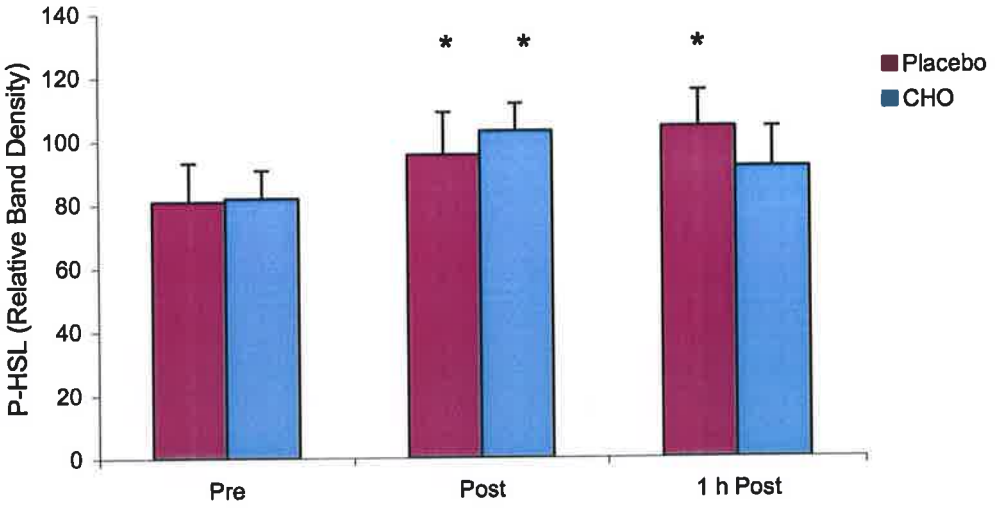


Figure 14: Hormone Sensitive Lipase Phosphorylation in Response to Exercise and Recovery for the Carbohydrate and Placebo Trials.

Data are presented as mean ± SE. * Significantly different from baseline ($p<0.05$)

Chapter 5: Discussion

Introduction

The aim of this study was to investigate the impact of carbohydrate ingestion on the regulation of fat oxidation following exercise. Exercise has been shown to have a strong influence in determining substrate utilization (21, 32, 131, 164). During exercise of low to moderate intensity, fat has been shown to be the dominant source of energy (33, 145, 165). The "crossover" concept described by Brooks (35, 166, 167) explains the shift in substrate oxidation towards a greater reliance on CHO that occurs as the exercise intensity increases above moderate. Exercise duration has also been demonstrated to influence fuel selection, with an increased relative contribution from fat oxidation during prolonged exercise. This is thought to be consistent with the decrease in the availability of CHO for energy as glycogen stores are used up (144).

There is a wealth of research available to support the increase in fat oxidation following exercise. From our observations, we can report that exercise does indeed increase fat oxidation in the post exercise period. This increase may be mediated by a number of factors including[U40]; 1) the increase in lipolysis as indicated by a rise in blood NEFA concentration and the suppression of plasma insulin levels following exercise, 2) the increase in AMPK phosphorylation which acts to partition fat away from re-esterification and storage and towards β-oxidation through its influence on ACC, MCD and GPAT, 3) the increase in phosphorylation induced inhibition of ACC and subsequent lowering of malonyl-CoA concentrations, releasing the inhibition on CPTI, 4) the likely suppression of GPAT activity indicated by the low plasma insulin levels and high phosphorylation of AMPK, and 5) the resynthesis of muscle glycogen in the post exercise period in preference over glycogen storage in the liver or oxidation in the

muscle

The Influence of Substrate Availability on Fuel Selection

The influence of substrate availability on fuel selection is also an area that has been widely researched since the proposal of the Glucose-FFA Cycle by Randle in the 1960's (145). In the glucose-FFA cycle, Randle et al. (145) suggested that an increased availability of NEFA to the working muscle leads to an increase in fat oxidation and a reduction in CHO oxidation. However, since its proposal, little evidence has been produced to support the existence of the cycle in human skeletal muscle, especially during exercise (164). Contrastingly, there is a growing body of research to suggest that a rise in CHO metabolism, through increasing exercise intensity or CHO ingestion, regulates fat oxidation (144, 168).

Our findings indicate that[U41]; 1) CHO ingestion inhibits lipolysis, indicated by the low levels of NEFA in the blood and is likely mediated by the anti-lipolytic action of insulin which was noticeably increased by CHO intake, 2) AMPK phosphorylation is lowered by glucose availability and uptake into muscle cells, which may influence the fate of NEFA by partitioning them towards storage and away from oxidation, 3) the phosphorylation induced inhibition of ACC is decreased, allowing for a rise in malonyl-CoA concentrations and a decrease in the activity of CPTI and 4) that despite the decrease in AMPK, ACC and HSL phosphorylation, CHO ingestion does not inhibit fat oxidation, indicated by the lack of a reduction in the rate of fat oxidation between trials and suggesting that other regulatory factors may play a role following CHO ingestion in recovery from exercise.

It must be noted that, although the outcome of CHO ingestion was a reduction of almost 30% in the contribution of fat to post exercise energy expenditure[U42], CHO intake following exercise did not result in a complete suppression of fat oxidation. It is thought that glycogen repletion is of such high metabolic priority that the majority of available glucose is shunted towards storage and fat used for energy (22, 23, 24, 25, 26).

In an attempt to gain further understanding of the regulation of fat oxidation in recovery and the effect of CHO ingestion, the following areas thought to partly regulate [U43]fat metabolism were investigated; 1) HSL and NEFA lipolysis, 2) FFA movement across the mitochondrial membrane and the influence of AMPK and ACC. Other regulatory pathways which may also exert an influential effect on fat metabolism were unable to be measured in the current study and so are not extensively discussed here.

Hormone Sensitive Lipase Regulation of Lipolysis and the Effect of AMPK

HSL is responsible for the lipolysis of NEFA from TG stored in adipose tissue and skeletal muscle (64, 66). It is phosphorylated and stimulated by epinephrine and contraction and dephosphorylated and inhibited by insulin (64, 96, 169). During exercise, insulin concentrations are low, reducing the inhibition of HSL (170). Coyle et al (30), reported insulin levels of 3-6 μU/ml at rest and during exercise in endurance trained men. In the present study, plasma insulin was decreased almost 3-fold on average below baseline, immediately after exercise in both the CHO and PLA trials. This occurred along with a corresponding increase in HSL phosphorylation in the muscle. We measured HSL phosphorylation on site Ser^{565} which is thought to be phosphorylated by AMPK (131). It has been proposed that AMPK acts to inhibit the HSL activation induced by β-adrenergic agents (131). Watt et al. (63, 64) reported an increase in HSL

activity in skeletal muscle from 1.67 ± 0.13 mmol·min^{-1}·kg^{-1} at rest to 2.06 ± 0.31 mmol·min^{-1}·kg^{-1} following exercise at 70%$\dot{V}O_{2peak}$ which was eliminated when AMPK activation was high due to low muscle glycogen levels or due to incubation with AICAR. The authors were led to the conclusion that β-adrenergic stimulation of HSL activity in skeletal muscle can be overridden by AMPK activation of HSL on Ser^{565} (135). There is an apparent contradiction in this conclusion as AMPK is employed to enhance energy producing pathways and by inhibiting HSL activity, it would in fact be eliminating energy provision from lipolysis (135).

We found that AMPK phosphorylation was increased following exercise by 1.3-fold on average. Although we did not measure AMPK activity directly, AMPK phosphorylation and ACC phosphorylation are thought to closely parallel AMPK activity (134). Our results are consistent with those of Roepstorff et al. (76) who reported a mean increase of 1.17-fold in HSL activity during exercise in both low and high glycogen trials, despite distinctly higher AMPK activity at 30 min during the low glycogen trial. It is thought that AMPK may not be a primary regulator of HSL activity during exercise as phosphorylation of HSL on Ser^{565} has been demonstrated without a corresponding increase in HSL activity (135). In any event, there is evidence to refute the suggestion that AMPK inhibits the β-adrenergic stimulation of HSL activity as the results of the present study demonstrate.

During recovery in the PLA trial, insulin levels remained at baseline level. In accordance with this suppression of serum insulin, HSL phosphorylation remained high and was still higher than baseline after 60 min of recovery. In contrast, CHO ingestion following exercise caused serum insulin levels to increase noticeably (11.5-fold) above

baseline. Accordingly, HSL phosphorylation at 60 min recovery was not different to that at baseline in the CHO trial. AMPK phosphorylation at 1 h post exercise was also not different to that at baseline in either trial. CHO ingestion caused AMPK phosphorylation to decrease more [U44]at this time point than placebo ingestion ($p < 0.05$). Lipolysis has been shown to be suppressed following CHO ingestion during exercise, to the extent that fat oxidation is limited (11, 171, 172). Horowitz et al. (55) reported a close matching of whole-body lipolysis and fat oxidation with an increase in lipolysis raising fat oxidation rates from 3.1 $\pm$ 0.3 to 4.0 $\pm$ 0.4 $\mu U \cdot kg^{-1} \cdot min^{-1}$. In the present study, CHO ingestion following exercise resulted in a reduced contribution of fat to energy expenditure (%EE). This is probably due partly to a decreased availability of NEFA as a result of low levels of HSL phosphorylation and an increase in the glucose available for metabolism. NEFA levels in the blood were distinctly reduced after 1 h of recovery in the CHO trial. At this time point, NEFA were lower than they were before exercise and were also lower than at 60 min post in the placebo trial.

In conjunction with skeletal muscle, adipose tissue and the liver play an important role in regulating the levels of NEFA and glucose in the blood. The HSL mediated lipolysis of triglycerides from adipose tissue is influenced by changes in insulin[U45] along with alterations in the concentration of epinephrine. It is likely that the large increase in insulin levels caused by CHO ingestion had an inhibitory effect on adipose tissue lipolysis (173). Unfortunately epinephrine concentration was unable to be measured in this study. CHO ingestion also exerts an effect on glucose production in the liver. When blood glucose levels are high, the liver stores glucose as glycogen to release later when the need arises. The high insulin levels arising from CHO ingestion also inhibit hepatic glucose production from gluconeogenesis and promote glycogen synthesis (173).

The Impact of Carbohydrate Ingestion on AMPK and Acetyl-CoA Carboxylase

An increase in glucose availability impacts on the activity of AMPK which in turn affects the transport of FFA across the mitochondrial membrane. AMPK responds to changes in fuel availability and is intimately linked to the control of fuel metabolism (68). During exercise, muscle glycogen stores were depleted to approximately 24% of their pre-exercise levels. The resultant change in the AMP/ATP ratio activated AMPK which in turn phosphorylated and deactivated ACC (68). We have shown ACC phosphorylation to be increased above baseline immediately after exercise. ACC is responsible for the formation of malonyl CoA, a potent inhibitor of fat oxidation (56, 174). The inactivation of ACC following exercise results in a decline in the levels of malonyl CoA. Reduced malonyl CoA levels result in a release of the inhibition of CPTI, the rate-limiting enzyme responsible for transporting LCFA into the mitochondria for β-oxidation (135, 175). Glucose ingestion causes changes in AMPK activity in the opposite direction (125, 141). Increased glucose availability following CHO ingestion raises the concentration of malonyl CoA by decreasing the activity of AMPK and by increasing the cytosolic concentration of citrate, an allosteric activator of ACC (125, 141). At 60 min recovery, ACC phosphorylation was lower [U46]in the CHO than in the PLA trial ($p < 0.05$), in compliance with AMPK phosphorylation. Rasmussen et al. (135) reported a 2.7-fold increase in malonyl-CoA concentration in six healthy subjects following physiological hyperglycemia and hyperinsulinemia[U47]. Although we were unable to directly measure malonyl-CoA concentration or activity, it is possible that this would have been increased in the CHO trial in accordance with the results on AMPK and ACC.

The major finding of the present study was that exercise resulted in an increase in the phosphorylation of AMPK, ACC and HSL while CHO ingestion acted to suppress

each. It has been previously suggested that AMPK may not be a key regulator of HSL activity. In this case, it is possible that the effect of contraction on HSL occurs at Ser^{600} and is mediated by PKC, partly via activation of ERK (64, 156, 169). The low insulin levels, high NEFA levels and enhanced rate of fat oxidation during recovery in the PLA trial indicate that HSL promoted FFA availability by skeletal muscle lipolysis. AMPK also inactivates ACC leading to a decrease in malonyl CoA concentrations and a release of the inhibition on CPTI. Rasmussen et al. (127) demonstrated a decrease in malonyl CoA levels and ACC activity in rat skeletal muscle for at least 90 min following 30 min of submaximal running. In the present study, ACC phosphorylation was increased following exercise in both trials indicating a deactivation of the enzyme. This is consistent with previous findings of an increase in fat oxidation rates following exercise (176). Horton et al. (177) also reported increased relative contribution of fat oxidation to total energy expenditure following exercise from 43.7 $\pm$ 2.1% of total EE during exercise to 58.3 $\pm$ 1.2% during recovery in men and from 50.9 $\pm$ 1.8% to 59.6 $\pm$ 1.7% in women. It must be noted here however, that there is a body of research to suggest that regulation of CPTI may be more complicated than regulation by malonyl-CoA alone. Some studies have reported no decrease in malonyl CoA levels during prolonged submaximal exercise despite elevated FA oxidation (132, 133, 134). One theory that has been proposed is the existence of a pool of malonyl-CoA in the mitochondria close to CPTI and separate to the pool located in the cytoplasm (132, 133, 134). This further complicates the interpretation of findings related to measurements from whole tissue.

Taken with our observations of a decrease in the contribution of fat and an increase in the contribution of CHO to overall energy expenditure post exercise, it is likely that CHO ingestion worked to lower fat metabolism during recovery from exercise

in the CHO trial. The observed rise in blood glucose levels resulted in a significant increase in plasma insulin levels. Insulin is the key anti-lipolytic hormone in adipose tissue and is thought to exert an inhibitory effect on HSL in skeletal muscle (68). The reduction in NEFA levels at 60 min recovery in the CHO trial below those of the PLA trial indicates an inhibition of lipolysis and is supported by our findings on HSL phosphorylation. HSL phosphorylation 1 h after exercise was significantly increased above baseline in the placebo trial but not in the CHO trial.

The considerable increase in blood glucose levels following CHO ingestion also worked to dephosphorylate and inactivate AMPK. As a result, ACC phosphorylation was decreased and activation increased. Enhanced ACC activation probably resulted in a rise in the concentration of malonyl CoA and an inhibition of CPTI and transport of FFA into the mitochondria. It is likely that CHO ingestion in recovery from exercise reduced fat metabolism through the coordinated effects of decreased FFA availability secondary to decreased adipose tissue lipolysis and fat oxidation at the muscle (148).

Carbohydrate Intake and the Fate of Free Fatty Acids

Following glycogen depleting exercise, repletion of glycogen stores takes priority and the majority of available glucose partitioned towards storage. As a result, fat is the primary fuel used for oxidation (4, 32, 178). Kuo et al. (26) reported RER values of 0.76 $\pm$ 0.01 in men and 0.78 $\pm$ 0.01 in women following 1 h of exercise at 65% $\dot{V}O_{2peak}$. This is comparable with our findings of an RQ of 0.82 $\pm$ 0.02 post exercise without CHO ingestion. CHO ingestion following exercise allows more glucose to be available for both storage and oxidation. Consequently, fat oxidation is reduced and FFA esterification

and storage as TG is enhanced (171, 179). Igal et al. (157) overexpressed mtGPAT in Chinese hamster ovary (CHO) cells and reported findings of a 1.9-fold increase in FFA incorporation into triacylglycerol. mtGPAT is involved in TG synthesis and has been reported to be increased in response to a rise in plasma insulin levels and deactivated by AMPK (21, 96, 157). Muoio et al. (156) reported a 62% increase in TG synthesis in cultured rat hepatocytes when insulin was present. The authors also reported a 50% decrease in TG synthesis when the same cells were incubated in AICAR. In accordance with this, AICAR also resulted in a 22-34% decrease in the activity of GPAT. Although we were unable to measure mtGPAT in the present study, it is likely that the decrease in AMPK phosphorylation and the sharp increases in plasma insulin resulted in the stimulation of mtGPAT and the promotion of FFA esterification and TG storage. The failure of CHO to completely suppress fat oxidation during recovery however, suggests that factors other than regulation by AMPK, ACC and HSL may play a role in influencing the fate of NEFA following CHO ingestion after glycogen depleting exercise.

Conclusion

The results of the present study indicate that an intricate interplay of metabolic components regulates fat oxidation in human skeletal muscle during and following exercise. Although fat oxidation post exercise was suppressed by glucose intake, it was not inhibited completely, indicating that following glycogen depleting exercise, repletion of muscle glycogen stores may be of such high metabolic priority that the majority of available glucose is shunted towards storage and so fat is required for energy needs.

Recommendations

Unfortunately we were unable to measure directly the activity of AMPK, ACC or HSL. While AMPK and ACC phosphorylation have both been shown to closely parallel AMPK activity, there is some controversy surrounding the effect of AMPK on HSL phosphorylation. For these reasons, our results on HSL phosphorylation must be interpreted with some caution. It is recommended that future research focus on measuring both phosphorylation and activity of AMPK, ACC and HSL. It is also advisable to measure the phosphorylation of HSL at its other serine sites, which it is thought may be more specifically related to HSL behavior during exercise. Although it has been proven to be quite difficult to measure the activity of GPAT in human skeletal muscle due to the low concentrations of the enzyme, future research concentrating on the regulation of fat oxidation after exercise would benefit a great deal from these results as they may help in understanding the fate of NEFA following the ingestion of CHO. Other variables which may be useful in fully understanding the regulation of fat oxidation and which it may be advisable to analyze include, malonyl-CoA, CPTI, MCD, DGAT and, in light of the recent studies by Spriet and his co-workers, CD36. The majority of published research focuses on CHO ingestion before and during exercise rather than in recovery from exercise. Future research is recommended to examine the influence of CHO intake post exercise on fat oxidation in light of our findings of a failure of CHO ingestion to suppress fat oxidation despite lowered phosphorylation of AMPK, ACC and HSL.

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Appendices

Appendix A: Data Collection Sheets

Impact of CHO ingestion on regulation of fat oxidation (visit 1)

DATE ☐☐/☐☐/☐☐☐☐ **SUBJECT ID** ☐☐/☐/☐/☐☐☐

CONTACT DETAILS)

Last name: _____ **First name:**
_____

Date of birth:_____ **Age** _____

Address:
_____
__

_____

Mobile _____ Work:_____
Home:_____

Email Address: _____

During the last 6 months, on average how many times per week did you exercise vigorously?

_____ times per week

What type of exercise? _____

Physical activity within 24h? Yes No

MEDICAL HISTORY (PHYSICIAN ADMINISTERED)

Height_____cm. Weight _____kg. Blood Pressure ___ / ____ Pulse____

SECTION A: Hospitalisation Record

1. Were you hospitalised due to any of the following?

a) Pain in the calf on walking YES_____ NO_____
Specify_____

b) Obstruction or thrombosis of blood vessels YES_____ NO_____
Specify_____

c) Palpitation YES_____ NO_____
Specify_____

d) Heart failure YES_____ NO_____
Specify_____

e) Stroke YES_____ NO_____
Specify_____

f) Other causes of loss of consciousness YES_____ NO_____
Specify_____

g) Tumours YES_____ NO_____
Specify_____

h) Operations YES_____ NO_____
Specify_____

SECTION B: Diabetes--Excluding Criteria

1. Have you ever had diabetic coma? (explain)
YES_____NO_____
If "yes," have you had it more than one time?
YES_____NO_____

2. Have you ever had hypoglycaemic crisis (explain) which required emergency measures?
YES_____NO_____

SECTION C: Hypertension

1. Do you take any of the following medicines for high blood pressure:

ACE Inhibitors	YES_____	NO_____
Ganglion blockers	YES_____	NO_____
Hydralazine	YES_____	NO_____
Calcium Channel Blocker	YES_____	NO_____
Other_____	YES_____	NO_____

SECTION D: Effort Chest Pain

1. Have you ever had any pain, pressure, heaviness, or discomfort in your chest? YES_____ NO_____
   If "yes", then where? (record area mentioned)
   _____

2. Do you get any of the above (pain, pressure, etc.) while you walk uphill or fast? YES_____ NO_____

3. Do you get it while you walk at an ordinary pace on the level YES_____ NO_____

4. Do you get it:

After a meal?	YES_____	NO_____
When emotionally excited?	YES_____	NO_____
At other times?	YES_____	NO_____

   Specify_____

IF NO TO 1-4 THEN SKIP TO SECTION E

5. What do you do when you get it?
   Step or slow down Take medicine Nothing Other
   Specify_____
   If "Stops":

6. If you stand still, what happens?
   Relieved_____
   No relieved_____
   If "relieved" 6a. How soon?
   10 min or less_____
   more than 10 min_____

If "Take medicine:"

7. What medicine do you take?_____
   a. Does it help YES_____ NO_____

   How soon? 1-7 minutes_____More than 7 minutes_____

8. Did you see a doctor for this pain? YES_____ NO____
   If "yes":
9. What did he/she say it was?
   _____

SECTION E: Myocardial Infarction with Hospitalisation

1. Have you ever had a severe pain or pressure across the front of your chest, last for 1/2 hour or more?

YES_____ NO_____
If "NO"--skip to 4
If "YES"--

1. Were you hospitalised because of this pain?

YES_____ NO_____
If "NO"--skip to 3
If "YES"--
2a. For how long? 3 days or less_____
3-20 days_____
21 days or more_____

3. What were you told by your doctor about your disease?
   _____

2. Have you ever been hospitalised because of a heart attack?

YES_____ NO_____

PHYSICAL EXAMINATION

GENERAL

Subject looks: Healthy____ Not healthy___ Very ill____

Subject: Coughs persistently_____
Orthopneic_____
Very pale_____

HEAD AND NECK

None of the following:

arcus senilis_____
blindness_____
exophthalmos_____
distended jugular veins, sitting_____
anisocoria_____
enlarged lymph nodes_____
enlarged thyroid:
- diffuse _____
- nodular _____
- single nodule _____
- xanthelasma _____

other head and neck abnormalities_____

CHEST AND LUNGS

None of the following (please specify):

barrel chest_____
dyspnea, sitting_____
expiratory rales, or rhonchi_____
inspiratory rales or rhonchi_____
wheezes_____
diminished breath sounds_____
other chest and lung abnormalities_____
specify_____

HEART

None of the following (please specify):

apex displaced lateral to 10 cm from MSL_____
abnormal precordial thrust_____
gallop rhythm present_____
arrhythmia present_____
heart considered enlarged_____
thrill palpable
systolic_____diastolic_____basal_____apical_____

left sternal border_____
distinctly abnormal tones or pathological sounds_____

RESTING ECG
Descriptive Analysis:

Rate: _____**bpm**
Rhythm: _____
Arrhythmias _____
_____
_____

Clinical Impression:

_____
_____
_____
_____
_____
_____
_____
_____
_____

MURMURS
None of the following:

Grade murmurs: 1 Barely audible 2 faint 3 moderate 4 loud
5 very loud 6 loudest possible

systolic murmur present_____grade 1 - 6_____

location
sternal border _____
apical _____
2nd on left intercostal space _____
2nd on right intercostal space _____
other _____

Pitch quality-type _____
Transmission _____
More than 1 systolic murmur _____
Murmur(s) considered organic _____
Murmurs considered nonsignificant _____

ABDOMEN
None of the following:

Hepatomegaly _____
Splenomegaly _____
Other palpable measures _____
Possible abdominal aneurysm _____
Other abdominal findings _____

EXTREMITIES
None of the following:

Deformities _____
Edema _____
Other findings _____

Specify those relevant for perip vasc. insuff. and impairment of gait

NEUROMUSCULAR
None of the following:

Romberg's sign _____
Ataxia, walking _____
Other neuromuscular findings _____

Specify_____

PHYSICIAN'S IMPRESSION

(Check the appropriate)

According to the patient's medical history and physical exam:

1. Classical angina pectoris is: present_____
   possibly present_____
   not present_____

2. The subject was hospitalized for, most probably, myocardial infarction _____

3. The subject was hospitalized for:
   a) Cardiac failure_____
   b) Diabetic coma, hypoglycemia crisis_____
   c) Mental disease_____
   d) Arrhythmia_____
   e) Cerebrovascular accident_____
   f) Peripheral arterial insufficiency_____

4. During the last 6 months, on average how many times per week did you exercise vigorously?

   _____ times per week

Physical activity within 24h?	Yes	No
Alcohol within 3 days?	Yes	No
Type 2 diabetes?	Yes	No
Taking any medication/drugs	Yes	No

If yes, what are you taking?_____

5. The subject should be excluded from the study because he receives the following drugs:_____

   _____

6. The subject should be excluded from the study due to the following conditions:_____

   _____

7. Other reasons for exclusion:_____

   _____

8. The following tests are indicated to determine his eventual participation:

_____

_____

9. Do you consume alcohol regularly?

YES ☐

NO ☐

How many units per week? _____

10. Do you smoke?

YES ☐

NO ☐

How many cigarettes a day? _____

11. According to the medical history and physical exam, does subject qualify for this research study?

Yes ☐

No ☐

Comments:

_____

_____

_____ _____

(Physician's signature) (Date)

CELL BLOOD COUNT

WBC

RBC

HgB

Hct

MCV

MCH

MCHC

Plt

LY

MO

GR

LY#

MO#

GR#

RDW

MPV

Body Composition

Subject _____ **Date** _____

 # 1:

Triceps _____ Suprailiac _____

Subscapular _____ Pectoralis _____

Abdominal _____ Thigh _____

Midaxillary _____

 # 2:

Triceps _____ Suprailiac _____

Subscapular _____ Pectoralis _____

Abdominal _____ Thigh _____

Midaxillary _____

Average:

Triceps _____ Suprailiac _____

Subscapular _____ Pectoralis _____

Abdominal _____ Thigh _____

Midaxillary _____

MAXIMAL EXERCISE TEST

Name_____ ID_____ Date & time_____

Supervised by_____

Protocol_____

Time	*Workload*	*HR*	*RPE*	*VO_2 (l/min)*	*VCO_2 (l/min)*	*VO_2 (ml/kg/min)*	*R*

Comments:

SUBMAXIMAL EXERCISE TEST

Name_____ ID_____ Date & time_____

Supervised by_____

VO_2 (l/min) corresponding to 75% $\dot{V}O_{2max}$_____ Estimated Workload _____

Time	*Workload*	*HR*	*RPE*	*VO_2 (l/min)*	*VO_2 (ml/kg/min)*

Workload corresponding to 75% $\dot{V}O_{2max}$_____

Comments:

Dietary Record Sheet

Please record below everything that you eat and drink (including snacks) over the course of the three days leading up to the experimental exercise trial, and an indication of the quantity (e.g. cup, medium bowl, slice, small portion etc). Ideally you should choose food that you know you can easily access on the days leading up to the second experimental exercise trials.

Time	***Food and Quantity***
Breakfast	
Lunch	
Evening	

EXERCISE TRIAL

Name________________________ ID____________________ Date & time___________

Supervised by________________________

VO_2 (l/min) corresponding to 75% $\dot{V}O_{2max}$____________ Estimated Workload _________

Time	*Workload*	*HR*	*RPE*	*VO_2 (l/min)*	*VCO_2 (l/min)*	*RQ*

Muscle Biopsy Form

Name _____ ID _____

Date _____ Physician _____

Muscle Biopsy No. 1:

Parameter	Before muscle biopsy	After muscle biopsy
Blood pressure		

Comments _____

Muscle Biopsy No. 2:

Parameter	Before muscle biopsy	After muscle biopsy
Blood pressure		

Comments _____

Muscle Biopsy No. 3:

Parameter	Before muscle biopsy	After muscle biopsy
Blood pressure		

Comments _____

Blood Collection Sheet

Name_____ ID_____ Date & time_____

Supervised by_____

Weight	
Glucose	
10% solution	

Stage	Event	Time	Comments
Baseline	Blood Sample		
Ex+15	Flush Line		
Ex+30	Flush Line		
Ex+45	Flush line		
Ex+60	Flush Line		
Ex+75	Flush Line		
End	Blood Sample		
End+15	Blood Sample		
End+30	Blood Sample		
End+45	Blood Sample		
End+60	Blood Sample		
End+75	Blood Sample		

Comments:

Blood Analysis Sheet:

Sample		Reading 1	Reading 2	Average
Baseline	Lactate Glucose			
End	Lactate Glucose			
End+15	Lactate Glucose			
End+30	Lactate Glucose			
End+45	Lactate Glucose			
End+60	Lactate Glucose			

Comments: _____

_____

_____

_____

_____

_____

_____

Appendix B: Table of Statistical Output

	CHO		PLA				
Variable	**Mean**	**SE**	**Mean**	**SE**	**t**	**df**	**P value**
Age (yr)	23.4	1.7					
Height (cm)	184.2	2.4					
Weight (kg)	78.8	2.8					
BMI ($kg \cdot m^{-2}$)	23.2	0.5					
Fat Mass (kg)	7.6	1.2					
Lean Body Mass (kg)	71.2	1.8					
Percent Body Fat (%)	9.4	1.1					
VO_2 max ($ml \cdot kg^{-1} \cdot min^{-1}$)	58.0	2.1					
VO_2 max ($l \cdot min^{-1}$)	4.6	0.1					
$\dot{V}O_2$ ($ml \cdot kg^{-1} \cdot min^{-1}$)	41.4	1.6	41.9	1.3	-.787	7	.457
Percent $\dot{V}O_{2max}$ (%)	71.5	1.8	72.5	1.8	-.857	7	.420
Energy Expenditure ($kcals \cdot min^{-1}$)	16.0	0.4	16.2	0.4	-.807	7	.446
Total Energy Expenditure (kcals)	1439	33	1459	39	-.792	7	.454
Post Ex EE ($kcals \cdot min^{-1}$)	1.95	0.07	1.84	0.11	1.688	7	.135
Post Ex RQ	0.87	0.02	0.82	0.02	2.791	7	.027
CHO Ox ($g \cdot min^{-1}$)	0.25	0.03	0.16	0.02	3.080	7	.018

Fat Ox($g \cdot min^{-1}$)	0.10	0.02	0.12	0.02	-1.935	7	.094
CHO%EE (%)	55.3	7.4	39.0	5.5	2.661	7	.032
Fat%EE (%)	44.7	7.4	61.0	5.5	-2.685	7	.033
Plasma Glucose ($mmol \cdot l^{-1}$)							
Baseline	4.79	0.19	5.01	0.09	1.154	7	.286
End of Exercise	4.62	0.22	4.58	0.18	-.243	7	.815
15 min Post Exercise	6.18	0.51	5.92	0.81	-.280	7	.788
30 min Post Exercise	8.47	0.69	4.60	0.14	-6.520	7	.000
45 min Post Exercise	8.34	0.49	4.50	0.14	-9.249	7	.000
60 min Post Exercise	7.57	0.53	4.41	0.14	-6.370	7	.000
Serum Insulin ($uIU \cdot mL^{-1}$)							
Baseline	4.75	0.75	3.63	0.44	-2.578	7	.037
End of Exercise	1.47	0.57	1.44	0.70	-.158	7	.879
15 min Post Exercise	13.66	3.18	4.26	1.1	-3.097	7	.017
30 min Post Exercise	35.55	7.46	3.14	0.4	-4.478	7	.003
45 min Post Exercise	49.49	5.56	2.98	0.52	-8.730	7	.000
60 min Post Exercise	54.85	13.74	2.38	0.48	-3.863	7	.006

NEFA (mmol·l^{-1})							
Baseline	0.81	0.10	0.70	0.07	-.907	7	.395
End of Exercise	1.40	0.23	1.43	0.18	.243	7	.815
15 min Post Exercise	1.89	0.28	2.17	0.27	1.272	7	.244
30 min Post Exercise	1.57	0.28	1.40	0.21	-.594	7	.571
45 min Post Exercise	0.81	0.15	1.25	0.18	1.971	7	.089
60 min Post Exercise	0.43	0.06	1.36	0.18	6.121	7	.000
Glycogen (mmol·kg^{-1}dry weight)							
Baseline	142.76	15.47	149.58	10.90	.368	7	.724
End of Exercise	33.82	7.80	36.31	12.12	.182	7	.861
60 min Post Exercise	48.61	9.11	58.29	11.10	.637	7	.544
Phospho-ACC (relative band density)							
Baseline	125.37	26.21	116.94	35.45	-.678	7	.519
End of Exercise	164.01	36.87	176.87	50.29	.347	7	.739
60 min Post Exercise	76.55	26.84	132.62	10.86	2.773	7	.028

Phospho-AMPK (relative band density)							
Baseline	167.59	37.35	141.82	35.06	1.616	7	.150
End of Exercise	201.55	47.80	200.30	53.20	.055	7	.958
60 min Post Exercise	121.76	21.15	105.12	16.93	2.961	7	.021
Phospho-HSL (relative band density)							
Baseline	81.90	8.89	80.86	12.19	-.147	7	.887
End of Exercise	103.02	8.56	95.47	13.41	-.749	7	.478
60 min Post Exercise	91.57	12.57	104.29	11.41	1.078	7	.317